JOURNAL OF AGRICULTURAL RESEARCH

VOL. 33 WASHINGTON, D. C., OCTOBER 1, 1926 No. 7

TOXICITY OF DIPYRIDYLS AND CERTAIN OTHER ORGANIC COMPOUNDS AS CONTACT INSECTICIDES[1]

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INTRODUCTION

Although the structural formula of nicotine has long been established, there is little prospect of an early accomplishment of the technical synthesis of this compound. However, compounds related to nicotine in structure may possess valuable insecticidal properties, so it was decided to prepare and test a number of these related compounds to determine their value as insecticides. As pyridine forms an essential part of the nicotine molecule it was selected as a starting point. A previous publication discussed the toxicity of some pyridine derivatives (*3*)[2] and referred briefly to the compounds which form the subject of the present investigation. It was found in these experiments that impure $\gamma\gamma$ dipyridyl was much more toxic than the purified substance, and it was later shown that these impurities consisted principally of isomeric dipyridyls. It is the purpose of this paper to report on the toxic value of these dipyridyls as contact insecticides. In addition, the toxicities of some other organic compounds used during the course of this work will be discussed.

THE CHEMICAL RELATIONSHIPS OF THE DIPYRIDYLS

In order to understand the chemical structure and properties of the dipyridyls, it is necessary first to consider pyridine, the compound from which they are derived. A number of drugs, including coniine, atropine, and cocaine, are derivatives of pyridine, and as a constituent of the nicotine molecule pyridine has more than the usual interest to the entomologist. Essentially, it is benzene with one of the CH groups replaced by nitrogen, and may be represented by the following graphic formula:

Pyridine differs from benzene in being distinctly basic and highly soluble in water, but resembles benzene in its great stability and in the unsaturated condition of its carbon atoms. Its toxic action as a contact insecticide seems to be low (*3*).

Under appropriate conditions, pyridine reacts with certain compounds to form substitution products in which the substituted atom or group displaces a hydrogen atom from pyridine and becomes

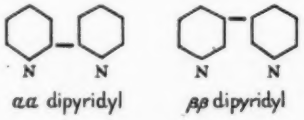
[1] Received for publication March 11, 1926; issued October, 1926.
[2] Reference is made by number (italic) to "Literature cited," p. 609.

attached to the pyridine ring. There are five carbon atoms in the pyridine ring which may be involved in this way; and their positions are generally designated as alpha (α), beta (β), gamma (γ), alpha' (α'), and beta' (β') according to the following scheme:

The dipyridyls are derivatives of pyridine composed of two pyridine molecules linked together with the elimination of one atom of hydrogen from each molecule. When metallic sodium reacts with pyridine at room temperature, a blue compound, sodium dipyridine, is formed (*4*). Sodium dipyridine $(C_5H_5N)_2Na$ has two pyridine molecules linked together and one sodium atom attached to the nitrogen atom of one of the pyridine rings.

Oxidation of sodium dipyridine with moist air results in the formation of $\gamma\gamma$ dipyridyl, the sodium atom and two hydrogen atoms being removed. Careful regulation of the temperature during the preliminary sodium-pyridine digestion, followed by oxidation (with dry air or oxygen) of the sodium dipyridines thus formed, leads to the production of an oil which contains chiefly $\alpha\alpha$, $\beta\beta$, $\beta\gamma$, and $\gamma\gamma$ dipyridyls. This crude dipyridyl oil, from which most of the $\gamma\gamma$ dipyridyl was removed, was used in the experiments described below. The other dipyridyls, the $\alpha\beta$ and $\alpha\gamma$ compounds, are also known and have been detected in the crude oil.

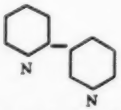
This completes the list of possible isomers in this series of compounds, $\beta\gamma$ dipyridyl being discovered and made available for the first time as a result of the chemical studies of these compounds (*4*). They may be represented by the following formulae; the formula of nicotine is added for comparison.



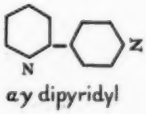
$\alpha\alpha$ dipyridyl

$\beta\beta$ dipyridyl

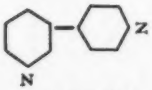
yy dipyridyl



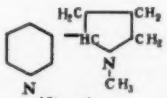
$\alpha\beta$ dipyridyl



$\alpha\gamma$ dipyridyl



$\beta\gamma$ dipyridyl



Nicotine

The constants and properties of the isomeric dipyridyls are given in Table I (*4*):

TABLE I.—*Constants and properties of the isomeric dipyridyls*

Compound	Melting point	Boiling point	Melting point of picrate	Solubility in water
	°C.	°C.	°C.	
αα dipyridyl	69.5	272.5	155	Only slightly soluble.
αβ dipyridyl	Liquid.	287–289	149.5	Insoluble.
ββ dipyridyl	68	296	232	Soluble in all proportions.
γγ dipyridyl	114	305	213	Soluble in hot water.
βγ dipyridyl	61	297	215	Very soluble in cold water, slightly soluble in hot water.
αγ dipyridyl	Liquid.	280–282	208	Insoluble.

The dipyridyls are solids or liquids at the ordinary temperatures. They have relatively high boiling points, and exhibit marked differences in their solubilities in water.

These compounds are basic but much weaker in this respect than pyridine. The αα and ββ dipyridyls are slightly volatile when a current of steam is passed through their solutions, but the other isomers obtained in this investigation are practically nonvolatile under these conditions.

There are certain similarities between nicotine and ββ dipyridyl that hould be emphasized at this point. Indeed, the similarity between this series of compounds and nicotine is sufficiently marked to have led chemists at one time to consider nicotine as a derivative of a dipyridyl (*1*). Nicotine is a β pyridyl-n-methylpyrrolidine; it consists therefore of a pyridine ring with a molecule of normal methylpyrrolidine attached at its β position. It resembles ββ dipyridyl further in its solubility in water, but is a much stronger base, has a considerably lower boiling point (247° C.), and is much more volatile in steam. βγ dipyridyl also resembles nicotine in regard to the linkage through the β position of a pyridine ring and in its solubility relationships.

PRELIMINARY EXPERIMENTS ON THE TOXICITY OF CRUDE DIPYRIDYL OIL

Studies on the toxicity of the dipyridyls as contact insecticides were begun in August, 1920. The first indication of toxic action appeared when a crude mixture, the result of an attempt to produce γγ dipyridyl, was found to be highly toxic to the bean aphis (*Aphis rumicis* L.) at a concentration of 1 per cent of the mixture.[3] Experiments soon demonstrated that purified γγ dipyridyl was only slightly toxic, if at all, and that the mother liquor separated from it was even more poisonous to insects than the original mixture. These experiments were made with samples obtained from pure pyridine which had a boiling point of 115° C. An oily mixture resulting from the action of sodium upon the homologs of pyridine (boiling point from 140° to 150° C.) yielded an ether-soluble and water-soluble fraction, each of which was considerably less toxic than the crude oil from pyridine. An oil similarly derived from crude alpha picoline

[3] All percentages which refer to concentrations of solutions or mixtures are to be understood as grams dissolved or emulsified in 100 c. c. of the solution or emulsion, respectively.

also proved to be less poisonous than that from pyridine. From these experiments it seemed probable that a highly toxic compound or group of compounds existed in the oil derived from pyridine, but that the compounds derived from the higher pyridine bases by this reaction were much less toxic.

METHODS OF APPLICATION OF CRUDE DIPYRIDYL OIL

After a large number of preliminary experiments, a crude dipyridyl oil known to contain a variable mixture of $\alpha\alpha$, $\beta\beta$, and $\beta\gamma$ dipyridyls was prepared for the toxicity experiments. It was dark brown in color and contained a small quantity of an insoluble tarry substance, besides traces of other dipyridyls and unchanged pyridine. It was partially soluble in water, and had a specific gravity of about 1.12 at 30° C.

Mixtures[4] were prepared to contain a known weight of the dipyridyl oil in a volume of 100 c. c. Usually 0.3 per cent (moisture-free basis) of sodium-base fish-oil soap, prepared according to Quaintance and Siegler (*2*), was added to increase the wetting and spreading of the solution.

The solutions were applied to the insects in two ways—by spraying and by submergence of the insects in them. In the spraying experiments the solutions of crude dipyridyl oil were applied with a small hand atomizer, special care being taken to cover all surfaces of the plant with the liquid. To obtain the approximate toxic concentration, it was sufficient to spray potted plants or portions of plants infested with aphids with a graded series of concentrations; the plants were kept fresh in a bottle which contained water. For more accurate results, a portion of an infested plant was placed in a bottle containing water, the opening was closed with cotton, and the bottle placed on a piece of paper. A barrier of adhesive material about the edges of the paper prevented the escape of the aphids. Eighteen or 24 hours after spraying the insects were counted and the percentage of dead determined.

A somewhat different procedure was followed in the submergence experiments. In these the insects were placed in a small dish, the solution to be tested was poured over them, and the dish was rotated gently for 1 minute, the purpose of this movement of the dish being to keep the insects in intimate contact with the solution. Then the solution was quickly decanted, and the insects were placed on dry filter paper to remove the surplus liquid. Afterwards they were placed in large beakers covered with muslin and supplied with food. The percentage of dead insects was determined within 18 to 24 hours after submergence.

INSECTS USED IN THE EXPERIMENTS

Six species of aphids, the adults of 1 species and the larvae of 2 species of Coleoptera, and the larvae of 2 species of Lepidoptera, were used in these experiments, as follows: The bean aphis (*Aphis rumicis* L.) on dwarf nasturtium (*Tropaeolum majus*), the spinach aphis (*Myzus persicae* Sulz.) on cabbage, the pea aphis (*Illinoia pisi* Kalt.) on garden peas, the turnip aphis (*Rhopalosiphum pseudobrassicae* Davis) on turnips, the rosy aphis (*Anuraphis roseus* Baker) on apple, the apple aphis (*Aphis pomi* De Geer) on apple; and larvae

[4] All mixtures of crude dipyridyl oil and soap contained the oil partly in solution and partly emulsified

and adults of the Colorado potato beetle (*Leptinotarsa decemlineata* Say), larvae of the three-lined potato beetle (*Lema trilineata* Oliv.), larvae of the Mediterranean flour moth (*Ephestia kuehniella* Zell.), and larvae of the silkworm (*Bombyx mori* L.).

SPRAYING EXPERIMENTS WITH APHIDS

Nasturtium plants infested with *Aphis rumicis* were sprayed with crude dipyridyl oil and nicotine. Figure 1 illustrates the comparative toxicity of the two compounds to this insect.

The points in Figure 1 represent actual experiments; the curves follow the mean values.

The curve for dipyridyl oil is derived from the following data:

Per cent concentration	Number of experiments	Number of aphids
0.02	10	1,380
.03	4	542
.04	10	1,459
.05	4	350
.06	4	396
.08	1	111
.10	1	105
.11	1	128

The curve for nicotine is based on the following:

Per cent concentration	Number of experiments	Number of aphids
0.005	2	296
.006	2	420
.007	2	329
.009	2	317

The asterisks in Figure 1 represent experiments in which the number of aphids killed was estimated. When the toxicity is high it is possible to make very close estimates of the percentage killed. Of course, when all the insects are killed the estimate is as exact as an actual count.

0.3 per cent sodium-base fish-oil soap was added to all the solutions used in these experiments.

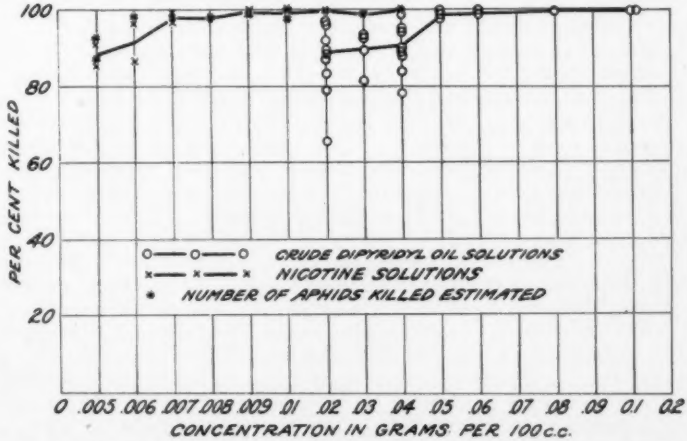


FIG. 1.—Comparison of the toxicity of crude dipyridyl oil and nicotine to *Aphis rumicis* on nasturtium plant (spraying experiments)

Crude dipyridyl oil killed more than 95 per cent of the aphids on nasturtium plants, at approximately 0.05 per cent concentration; and nicotine was equally toxic at about 0.007 per cent concentration. Tattersfield and Morris in a recent paper (*5*) give toxicity figures for nicotine which indicate an equivalent toxicity to *Aphis rumicis* with concentrations of 0.08 to 0.1 per cent, or about 12 to 14 times higher than the value given above. Aside from differences in the methods of application and the spreader used, those workers employed only the adult apterous agamic females in their experiments. All the work on *A. rumicis* mentioned above was done on adults and young of various ages as they occurred naturally on the nasturtium plants.

A number of spraying experiments were made on *Myzus persicae* which infested young cabbage plants. Crude dipyridyl oil at a concentration of 0.05 per cent gave 100, 98.4, and 95+ per cent mortality, respectively, in three trials, but at a concentration of 0.01 per cent many living aphids remained on the plants. The concentration necessary to kill 95 per cent or more of these aphids would therefore appear to lie close to 0.05 per cent. In a preliminary series of experiments the toxic concentration for nicotine lay between 0.007 and 0.01 per cent. Unless otherwise stated, 0.3 per cent sodium fish-oil soap was used in all solutions and mixtures. Its toxicity to *Aphis rumicis* has previously been given (*3*, *p. 8*).

Six spraying tests with crude dipyridyl oil involving 883 individuals of *Illinoia pisi* were made with the following results: In two tests at 0.05 per cent concentration, 86.5 and 91.7 per cent mortality; in two tests at 0.1 per cent, 97 and 100 per cent mortality; and in two tests at 0.69 per cent, 100 per cent mortality. Soap was added to these solutions as indicated above.

Turnip plants infested with 200 to 800 individuals of *Rhopalosiphum pseudobrassicae* were sprayed with crude dipyridyl oil and nicotine. In the nine tests the dilutions ranged from 0.05 to 1.1 per cent; the fish-oil soap concentrations were 0.1 and 0.3 per cent. Twenty-five one-hundredths per cent of the dipyridyl oil killed 95 per cent or more of the aphids. Under similar conditions nicotine required a concentration of about 0.05 per cent (seven experiments).

A series of nine experiments was made with the rosy aphis (*Anuraphis roseus* Baker) on apple twigs. The soap concentrations used were 0.6, 0.3, and 0.1 per cent. Since this aphid showed a rather high susceptibility to fish-oil soap solutions, 0.1 per cent soap was employed in most of the sprays. The concentrations of dipyridyl oil ranged from 0.05 to 0.2 per cent, the latter giving approximately a 95 per cent mortality. Two comparative tests were made with a commercial nicotine sulphate solution containing 0.05 per cent nicotine and 0.3 per cent fish-oil soap. In these tests 87 and 100 per cent of the aphids were killed. This dosage is close to the field recommendation of ¾ pint of nicotine sulphate containing 40 per cent nicotine in 100 gallons of water (i. e., 0.0375 per cent nicotine)

Portions of a small Siberian crab apple tree infested with *Aphis pomi* De Geer were sprayed with 0.2 per cent crude dipyridyl oil mixture containing 0.1 or 0.6 per cent fish-oil soap. One hundred per cent of the aphids were killed in the two tests. No experiments with nicotine were made; the field recommendation of nicotine

sulphate generally given for this aphid, however, is equivalent to 0.0375 per cent nicotine in a solution containing from about 0.3 to 0.5 per cent of moisture-free soap.

SUBMERGENCE TESTS WITH OTHER INSECTS

Larvae of the Colorado potato beetle were submerged in solutions of crude dipyridyl oil and nicotine, with results that are shown graphically in Figure 2. The submergence time in these experiments was one minute.

Each point on Figure 2 is the result of a single experiment involving 25 larvae. The curves follow the mean values. Fifty larvae were used in the tests with 3.4 per cent nicotine, and 75 larvae with 1.1 per cent dipyridyl oil. All other concentrations involved

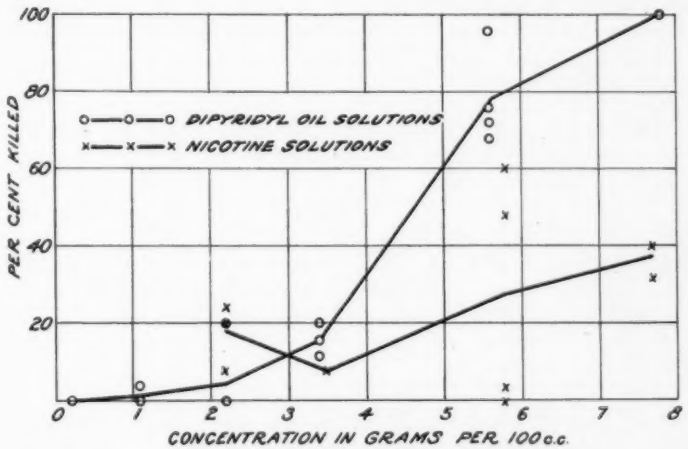


FIG. 2.—Comparison of the toxicity of crude dipyridyl oil and nicotine to larvae of *Leptinotarsa decemlineata* (submergence experiments)

100 larvae each. The weight of the larvae ranged from 2.6 to 4.9 gm. per lot of 25 individuals.

All solutions contained 0.3 per cent of sodium fish-oil soap. Five lots of 25 larvae each submerged in 0.3 per cent soap solution for one minute were unharmed.

At all concentrations, except possibly the lowest, dipyridyl oil was more toxic than nicotine. The solutions of nicotine which contained 3.4 per cent or more of nicotine paralyzed the larvae to such an extent that they always failed to recover, although in some cases they were alive 48 hours after treatment.

A few tests with adults of the Colorado potato beetle indicate that this stage of the insect is more susceptible to submergence in nicotine solutions than in crude dipyridyl oil mixtures. Two tests with 1 and 1.1 per cent nicotine solutions gave 100 per cent mortality (29 beetles). Three tests with 2, 2.5, and 2.6 per cent dipyridyl oil mixtures gave 100, 100, and 93.3 per cent mortality, respectively (37 beetles), whereas tests with 0.97 and 1.3 per cent solutions of dipyridyl oil

gave 80 and 28.6 per cent mortality, respectively (26 beetles). The 0.3 per cent soap concentration used in these experiments resulted in a mortality of 9.2 per cent (65 beetles). These experiments, while probably inadequate to establish the approximate toxicity, appear to indicate a higher value for nicotine than for dipyridyl oil.

When larvae of *Lema trilineata* were submerged in solutions of crude dipyridyl oil and nicotine under comparable conditions rather striking differences were observed, as Figure 3 shows.

The submergence time in these experiments was 1 minute. Each point in Figure 3 represents an experiment; the curves follow the mean values. Five hundred and eighty larvae of all sizes were used in 17 dipyridyl oil experiments, or from 22 to 45 larvae in each (average 34.1 per experiment). Seven hundred and forty-three larvae of all sizes were used in 23 nicotine experiments, or 16 to 40 larvae in each (average 32.3 per experiment). For all concentrations of nicotine above 2.7 per cent, commercial nicotine sulphate solution was

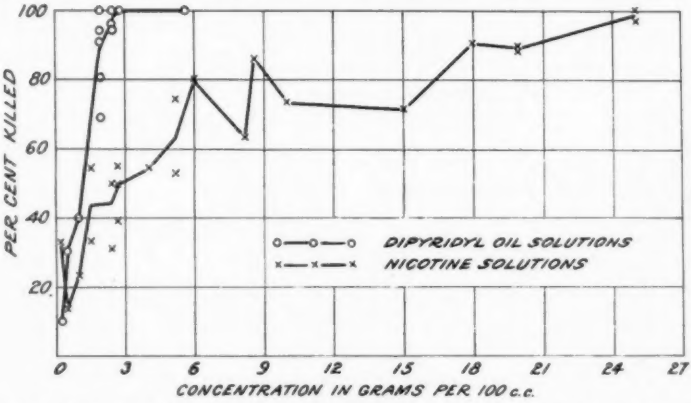


FIG. 3.—Comparison of the toxicity of crude dipyridyl oil and nicotine to larvae of *Lema trilineata* (submergence experiments)

used. It was diluted to contain nicotine, equivalent by weight to the percentage designated in Figure 3.

All solutions contained 0.3 per cent sodium fish-oil soap. In four experiments with a total of 118 larvae of all sizes the mortality produced by 0.3 per cent soap was 2.5 per cent.

The curve for dipyridyl oil is abrupt, the percentage of larvae killed rising to 97.4 at a concentration of 2.5 per cent of this mixture. The curve for nicotine rises more gradually and irregularly, and the unusually high concentration of 25 per cent is reached before more than 95 per cent of the larvae are killed. Many of the larvae treated with 4 and 5 per cent solutions of nicotine fed actively on potato foliage after treatment, whereas mixtures of dipyridyl oil of the same concentrations killed or incapacitated all the larvae treated.

Larvae of *Ephestia kuehniella* were submerged in mixtures of crude dipyridyl oil. Comparable tests were made with nicotine solutions also. The results of these experiments are shown graphically in Figure 4.

The submergence time in these experiments was 1 minute.

Each point in Figure 4 represents a single experiment; the curves follow the mean values. The dipyridyl oil experiments involved a total of 332 larvae 7 to 13 mm. in length; 24 to 36 larvae (average 27.7) were used in the individual experiments. For the nicotine experiments, 308 larvae 8 to 13 mm. in length were used, or 31 to 37 (average 34.2) in the individual experiments. All solutions contained 0.3 per cent sodium fish-oil soap. Experiments with this soap solution on 173 larvae gave the low toxicity of 2.9 per cent.

Under the conditions which prevailed in these experiments, dipyridyl oil was more toxic at all concentrations than nicotine. The highest (3.4 per cent) killed 93.3 per cent of the larvae, as compared with only 54.4 per cent for a 5 per cent solution of nicotine.

Larvae of the silkworm (*Bombyx mori*) were also used in submergence tests to ascertain the relative toxicity of crude dipyridyl

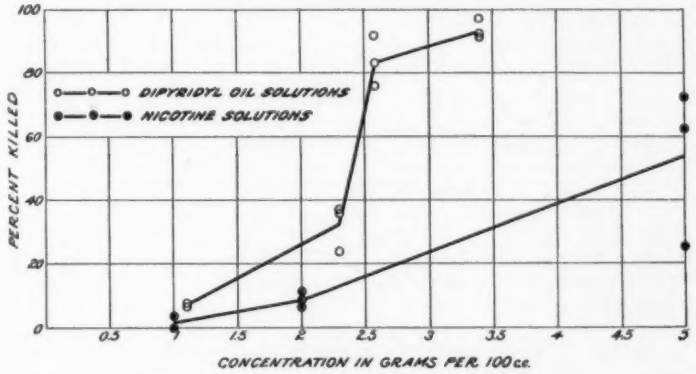


FIG. 4.—Comparison of the toxicity of crude dipyridyl oil and nicotine to larvae of *Ephestia kuehniella* (submergence experiments)

oil and nicotine. In 1923 the approximate toxic concentration was determined for both of these compounds by the treatment of small numbers of larvae with successive dilutions. Then 85 larvae 25 to 50 mm. in length were divided into four lots and submerged in a 1.5 per cent mixture of crude dipyridyl oil. From the four experiments an average kill of 97.6 per cent was obtained. In 1924, 100 larvae 30 to 40 mm. in length were treated in four lots of 25 larvae each with a solution of the same strength. The result was a mortality of 96 per cent, in close agreement with the previous determination. Under identical conditions nicotine was more toxic. Thirty-two larvae 25 to 50 mm. in length were used in four tests in 1923, a 0.01 per cent solution killing 96.9 per cent of the larvae. In 1924, of 100 larvae 35 to 50 mm. in length 95 per cent were killed by the same concentration. Some of the larvae of the 1924 experiments, however, probably were diseased, for of 103 control larvae treated with the 0.3 per cent soap solution 19.4 per cent died. Of the 31 control larvae in the earlier experiments (1923) 6.4 per cent were killed or died of disease.

In spite of the small number of larvae available for these experiments the results indicate, it is believed, a significant difference in toxicity in favor of nicotine.

The submergence tests show that the larvae of *Leptinotarsa decemlineata*, *Lema trilineata*, and *Ephestia kuehniella* are more susceptible to crude dipyridyl oil than to nicotine, but the reverse is true of adults of *L. decemlineata* and silkworm larvae. In view of these results, it seems possible that crude dipyridyl oil may prove to be more effective than nicotine for the practical control of some injurious insects.

EFFECT OF SPRAYS ON PLANTS

Sprays containing crude dipyridyl oil have been tested on a variety of plants, under both greenhouse and outside conditions. Nasturtium plants growing in the greenhouse in February and November, 1923, tolerated 0.1 per cent mixtures of crude dipyridyl oil, a concentration which lies well beyond the minimum toxic concentration (0.05 per cent) for *Aphis rumicis*. At 0.25 per cent concentration the injury to this plant was not severe. Cabbage plants in the greenhouse in April were not injured by mixtures containing 0.05 per cent; higher concentrations were not tried. One-tenth per cent mixtures burned slightly the tips of the fronds of Boston fern, whereas 0.04 per cent mixtures had a very slight effect or none on the young growth (February). Apple foliage and Coreopsis plants growing outdoors were not injured by 0.2 per cent mixtures of dipyridyl oil, and the young growing shoots of rose were unharmed by 0.25 per cent mixtures. All of the above mixtures contained 0.3 per cent sodium fish-oil soap as a spreader.

In every case in which aphid-infested foliage was sprayed, it was possible to kill the aphids without injury to the foliage.

INSECTICIDAL PROPERTIES OF THE CONSTITUENTS OF CRUDE DIPYRIDYL OIL

As stated above, the crude dipyridyl oil used in this investigation consisted largely of 3 isomers, $\alpha\alpha$, $\beta\beta$, and $\beta\gamma$ dipyridyl, with small quantities of other dipyridyls and other substances. It has already been shown that $\gamma\gamma$ dipyridyl has little toxic effect on *Aphis rumicis* (*3*, *p. 4*).

In order to study the insecticidal properties of the constituents of the crude oil, a number of preparations were made. Samples of $\alpha\alpha$, $\beta\beta$, and $\beta\gamma$ dipyridyl were obtained which gave constant melting points and were unquestionably of high purity. They were made up in mixtures containing fish-oil soap to compare with the other mixtures used in this investigation. Their toxicities to *Aphis rumicis* when applied as sprays are compared in Figure 5.

The experiments were made in groups of three for each concentration. In the $\alpha\alpha$ dipyridyl experiments from 707 to 2,249 aphids were used for each concentration; in the $\beta\gamma$ dipyridyl experiments, 818 to 1,097 aphids; in the $\beta\beta$ dipyridyl experiments, 851 to 1,103 aphids, except for the 0.05 and 0.1 per cent concentrations, where

349 and 206 aphids, respectively, were used. Each point in Figure 5 is an actual experiment; the curves follow the mean values of each group of experiments.

At the higher concentrations $\alpha\alpha$ dipyridyl appears to be a little more toxic than $\beta\gamma$ dipyridyl, a 0.6 per cent mixture of the former corresponding in toxicity with a 0.75 per cent mixture of the latter. The effect upon nasturtium foliage (under greenhouse conditions) is also different; 0.5 per cent mixtures of $\alpha\alpha$ dipyridyl applied in September and October caused only slight injury, whereas the same concentrations of $\beta\gamma$ dipyridyl applied in October caused severe injury. The tolerance of nasturium plants for $\beta\gamma$ dipyridyl was reached at approximately 0.1 per cent concentration.

The sample of $\beta\beta$ dipyridyl used in these experiments was synthesized from pseudophenanthroline by oxidation with potassium permanganate. As indicated in Figure 5, it proved to be less toxic than either $\alpha\alpha$ or $\beta\gamma$ dipyridyl, a 1 per cent solution killing only 93.8 per

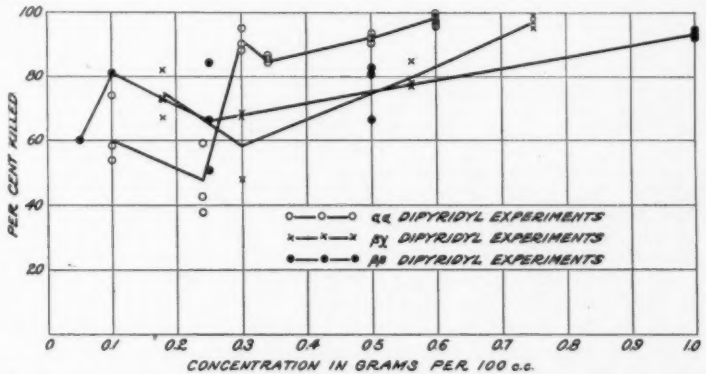


FIG. 5.—Comparison of the toxicity of $\alpha\alpha$ dipyridyl, $\beta\gamma$ dipyridyl, and $\beta\beta$ dipyridyl to *Aphis rumicis* (spraying experiments)

cent of *Aphis rumicis* in these tests. The tolerance of nasturtium plants for this compound in the greenhouse during August was of about the same order as that noted for $\beta\gamma$ dipyridyl.

The preparation of pure $\alpha\beta$ and $\alpha\gamma$ dipyridyls is under investigation at the present time. An impure sample of $\alpha\beta$ dipyridyl prepared from phenanthroline was much less toxic than crude dipyridyl oil.

From the above experiments it appears that the toxicity of crude dipyridyl oil is not dependent so much upon $\alpha\alpha$, $\beta\beta$, and $\beta\gamma$ dipyridyls as upon a compound or compounds present in the water-soluble fractions. This compound must have high toxicity to aphids, because several preparations which were unquestionably impure have killed about 90 to 95 per cent of *Aphis rumicis* at concentrations lying between 0.05 and 0.01 per cent, a close approximation to the toxicity of nicotine for this aphid. Work is being continued with the hope of isolating and identifying this compound.

INSECTICIDAL PROPERTIES OF OTHER COMPOUNDS

A number of other compounds were tested in the course of this investigation, and are included in Table 2.

TABLE 2.—*Spraying experiments against Aphis rumicis on nasturtium plants*

Compound	Concentrations tested, grams per 100 c. c.[a]	Concentrations necessary to kill 95 per cent or more of the aphids	Concentrations tolerated by nasturtium plants
Allyl alcohol	1, 2	>2	<2
Anisole [b]	1, 5, 10, 20	20	>1<5
Betahydroxypyridine	0.15	>0.15	>0.15
Betathionaphthol (commercial)	0.5,[c] 1 [c]	>1	<0.5
Carvacrol	0.25, 0.5, 1, 2	1	<0.25
Chloramine T	1, 5	>5	>5
Chlorocyclohexane	1, 5	>5	<1
Citral (technical)	0.25, 0.5, 1, 2	2	±0.25
Dimethylaniline	1, 5, 6	6	>1<5
Isonicotine	0.29, 0.69, 1, 1.5, 1.7	1.7	<0.29
Methylcyclohexane	7.5, 15	>15	±7.5
Paracymene	1, 5, 7	7	<1
Pyridinebetasulphonic acid	4.9	>4.9	<4.9
Pyrrolidone	0.1, 0.5, 1, 2	>2	0.5–1
Trioxymethylone	0.5, 1, 2	>2	>2
Urethane	1.2, 2.5, 5	5	1.2

[a] 0.3 per cent sodium fish-oil soap used as a spreader.
[b] Tattersfield, Gimingham, and Morris (*6, p. 250*) have found that anisole has little toxicity for *Aphis rumicis* at a concentration of 5 per cent.
[c] With sufficient NH_4OH to render it soluble.

Several of these are of special interest because of certain similarities to the dipyridyls or nicotine. Betahydroxypyridine and pyridinebetasulphonic acid, like nicotine, have the substituted group attached at the β carbon. Neither of these compounds, however, showed striking toxicity; like other sulphonic acids (*3, p. 6*), pyridinebetasulphonic acid was relatively nontoxic. Isonicotine resembles $\gamma\gamma$ dipyridyl in that it is formed by the linkage of two pyridine rings through the γ positions. It differs structurally from this compound in that one ring is hydrogenated to form a piperidine grouping. Although more toxic than piperidine (*3, p. 4*), it is still much less so than crude dipyridyl oil. None of the other compounds showed striking toxicity.

SUMMARY

A crude oil containing several isomeric dipyridyls and other substances has been prepared from pyridine and sodium. The physical properties and chemical relationships of the dipyridyls are discussed and their similarities to pyridine and nicotine pointed out.

In spraying experiments with six species of aphids, this crude dipyridyl oil was found to be highly toxic, but was surpassed in this respect by nicotine.

In submergence experiments, the crude oil was more toxic than nicotine to the larvae of two species of Coleoptera and one species of Lepidoptera, and less toxic than nicotine to the adult of one species of Coleoptera and to silkworm larvae.

Crude dipyridyl oil, in the present experiments, was not injurious to plants infested with aphids at concentrations sufficient to kill the aphids.

$\alpha\alpha$, $\beta\beta$, $\beta\gamma$, and $\gamma\gamma$ dipyridyls, which occur in the crude dipyridyl oil used in these tests, were not so toxic to *Aphis rumicis* as the crude oil itself; $\gamma\gamma$ dipyridyl is much less toxic than the other three compounds.

Several preparations from the crude oil proved to be highly toxic to *Aphis rumicis* on nasturtium plants. Although unquestionably impure, they approached closely the toxicity of nicotine for this aphid. Work is being continued on these preparations.

The toxicities of 16 other organic compounds tested during this investigation are given also (Table 2). Several of these are related to the dipyridyls or to nicotine. As compared with dipyridyl oil, they showed no appreciable toxicity.

Crude dipyridyl oil was more toxic than nicotine to certain insects used in this investigation, and it is possible that it may also prove more effective for the practical control of some injurious insects.

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AGE CLASSES OF WESTERN WHITE PINE PLANTING STOCK IN RELATION TO ASPECT OF PLANTING SITE IN NORTHERN IDAHO [1]

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INTRODUCTION

In the northern Rocky Mountain region there are vast areas of forest land denuded by fire, which will remain virtually unproductive for generations to come unless planted by hand with nursery-grown trees. After the first sweep of fire through the original stands of western white pine timber (*Pinus monticola*) on these lands, the forest in most instances started to come back naturally, but before recovery was complete fire came again and this time swept away all the small new trees and all sources of seed for future growth. Forest planting is the only practical means of making such land productive.

Because of the vast amount of forest planting to be done in this region, it is desirable that research point out the means by which it can be undertaken at smallest cost and with the best possible results in thrifty young trees. This paper is an account of a few experiments to that end, other studies with a bearing on survival of plantations being still under way.[2]

Two very important factors in forest planting are the aspect [3] or exposure of slopes used for planting and the class of stock employed, for these are factors which both singly and in combination have a definite effect on survival of the trees. In the experiments reported herein five classes of stock representing three different ages of trees were tested on each of four slopes. The exposure of the slopes was determined in each instance by averaging the compass readings on the plots of each series. The slope classed as northeast had no deviation from this direction and the northwest, east, and west did not vary greatly, the first being 14° north of due northwest and the last two 8° north of east and 2° south of west, respectively.

The topography was such that the four aspects studied could be found close together, each with a gradient of 25° to 30° and at practically the same elevation, 3,350 to 3,700 feet. It is essential in work of this kind to arrange test plots so as to minimize irregularities in uncontrolled influences, such as differences in the competing vegetation, variation in the direction of slope, and changes in the quality of soil within plots and between plots. The soil, for instance, is often deeper and more favorable for plant life near the base of a slope than higher up. In these experiments the plots on each slope ran parallel to each other up and down the slope, each containing 600 trees of a certain age class set in 8 rows. Because of the uniformity of the

[1] Received for publication Apr. 1, 1926; issued October, 1926.

[2] The experiments on which this report is largely based were started by E. C. Rogers near Wallace, Idaho.

[3] "Aspect," as used by foresters, denotes the direction toward which a slope faces.

sites as a whole and the narrowness of the plots used, there is every reason for confidence in the results obtained.

The slit method of planting was used. This method is rapid and requires but one man for the entire operation, the soil being worked with a short-handled mattock and the seedlings planted with the free hand.

The crew for the experimental plantings consisted of a foreman, eight planters, and a packer. Each planter set out one row of trees in each plot, so that the method of planting might be considered uniform. To make later observations and records easier, each plant was marked with a serial number on a white-tipped stake.

The planting was done in the spring of 1915 with age classes of nursery stock designated as 2–2, 1–2, 2–1, 2–0, and 1–1.[4] At the end of the dry period in the fall of 1915, 1916, and 1917, all plants were examined, the surviving plants were counted, and the current stem-height growth of 200 plants in each plot was measured to the nearest tenth of an inch. The measured plants were chosen from all parts of the plot, upper as well as lower ends, the selection of 25 plants from each row insuring an equal representation of the work of each planter. As nearly as possible, the same plants were measured each year. Changes in the list of trees measured the first year were made only where necessary in order to confine the observations on growth to normal, uninjured plants. This was done because mechanical injury bears no obvious relation to the age class of the trees or to the exposure of the planting site.

SURVIVAL BY AGE CLASSES

A study of the percentage of planted trees surviving through three seasons, as given in Table 1, makes apparent certain relationships.

TABLE 1.—*Survival percentages of western white pine planting stock by age classes, aspects, and seasons* [a]

Age class	Northwest			Northeast			East			West			Average		
	1915	1916	1917	1915	1916	1917	1915	1916	1917	1915	1916	1917	1915	1916	1917
2–2	100.0	99.4	99.1	98.7	98.6	97.6	99.2	98.3	98.2	97.3	93.2	87.4	98.8	97.4	95.6
1–2	99.5	98.8	98.8	99.8	98.7	99.0	99.4	98.8	98.4	95.9	92.2	80.7	98.7	97.1	94.2
2–1	98.4	96.7	95.8	99.0	97.6	96.7	98.4	96.6	95.9	95.0	87.9	77.4	97.7	94.7	91.4
2–0	97.5	96.3	94.6	98.4	96.0	91.7	99.3	98.6	97.5	91.0	80.5	69.4	96.5	92.8	88.3
1–1	97.6	95.0	93.5	96.1	90.1	88.0	95.1	90.8	88.1	83.8	75.4	63.1	93.2	87.8	83.2
Average	98.6	97.2	96.4	98.4	96.2	94.6	98.3	96.6	95.6	92.6	85.8	75.6	97.0	93.9	90.5

[a] Trees planted in May, 1915, on the Placer Creek area near Wallace, Idaho.

It may be seen from the average survival for all aspects that 2–2 stock was most successful, with 1–2, 2–1, 2–0, and 1–1 following in descending order. This order held for the favorable northwest aspect, and even more clearly for the west aspect, where survival in general was lower. On the slopes facing northeast and east the 2–2

[4] As readers familiar with nursery practice will know, this terminology indicates the number of years spent by the plant in the seed bed and in the transplant bed. For example, the 2–2 stock remained two years in the seed bed and two years in the transplant bed, and was 4 years old at the time of field planting. Similarly, 1–2 stock and 2–1 stock were both 3 years old, but the former was transplanted at 1 year of age the latter at 2 years. The 2–0 stock was seedlings 2 years old used in the field without previous transplanting.

stock yielded first place to the 1–2 stock, but only by small margins. The 1–1 stock was poorest on all aspects.

This similarity of the relative survival of age classes for different aspects gives added meaning to the figures for average survival as an indicator of quality of stock. The intrinsic qualities that enable one class of stock to survive better than another have not been definitely determined. Differences in survival, however, are thought to arise not only from variation in size and vigor as a result of difference in age, but also from variation in anatomical proportion resulting from different cultural treatment in the nursery. Thus 1–2 and 2–1 stock or 2–0 and 1–1 stock, although the same age, differ anatomically because of the different times at which they were pruned and transplanted in the nursery. The survival of 3-year-old trees was intermediate between the 2-year-old and 4-year-old trees, suggesting a general tendency toward higher survival with increased age. This tendency is not proved, however, because in this study the influence of age can not be separated from that of class of stock.

Aspect of planting site is as important a factor in survival as is class of stock, for reforestation of any area can be successful only when growing conditions there meet the requirements of the trees planted. Severity of site, as indicated by differences in survival, also emphasized the differences attributable to quality of stock. The west slope sites were clearly the most severe. The other, more moderate sites, were about equally favorable except that mortality was slightly less on the northwest slope than on the other two.

Assuming that all readily avoidable causes of death were eliminated from the tests, the above comparison on the basis of numbers of trees surviving gives a conception of relative values of classes of stock and aspects of planting site. Such an assumption, however, would not be justified in the absence of observations on the causes of mortality. For this reason all dead trees were dug out and inspected at the annual fall examination.

For most of the trees no reason for death other than drought could be detected, but occasional evidence of other causes was found. Some roots had been pruned too short, some trees had bark stripped from their roots, and others had scraped stems. These three causes can be kept at a minimum by careful handling of trees and the culling out of injured individuals. Shallow planting, whether resulting from roots cut too short or from trees set with their roots in poor positions, caused death because the surface layers of soil dried out most rapidly. Deep planting may have resulted in poor aeration for roots and may have buried part of the tops. Plants set under large fallen logs had a poor chance to live. Even after heavy September rains it was noticed that the soil in such places was dust-dry and often used by grouse for wallows. Still more fatal was the planting in what seemed to be fairly deep soil on the root mass of wind-thrown snags.

The most frequent cause of death listed other than drought was sliding soil on steep slopes. This is not to be confused with the rapid and much more destructive mass movement of soil, logs, and other débris caused by one of the snow slides common in the region, but is rather a creeping of soil, small rocks, and other surface litter from above the trees downward, usually caused by rain and melting snow. The sliding soil has a tendency to fill up the slight depression formed

by planting and to bury the trees. Death of trees resulting from covering by sliding soil naturally fell heaviest on the youngest and shortest-stemmed trees, the 1–1 stock.

These younger trees seemed also to have been more subject to stem injury from tamping. The older trees were probably bruised as often, but were better able to withstand the injury. Similarly, by far the largest number of dead trees showing evidence of either mechanical injury or poor planting were on the west-facing slope. Such unfavorable conditions were probably as frequent on other aspects, but there greater moisture enabled the trees to live in spite of adverse conditions.

Less than 1 per cent of the trees planted, or 10 per cent of those which died, were affected by the above-mentioned unfavorable conditions, leaving 90 per cent of the deaths attributable only to drought. As has been indicated, the older plants usually survived better than the younger and the transplants better than the seedlings.

The conclusion that transplants are hardier than seedlings is corroborated by the results of small tests of age classes at other points. In one of these tests at Priest River, Idaho, in the spring of 1915 where 2–0 and 1–2 western white pine stock were planted on a flat and on a northeast slope, the transplanted stock (1–2) yielded higher survival in both situations. In another test near Haugan, Mont., in the fall of 1922, parallel trials of 3–0 and 1–2 white pine stock on a northwest slope yielded higher survival again for the transplanted (1–2) stock. Also a plantation on a northeast slope in the fall of 1924 resulted in higher survival for 1–2 transplants than for 3–0 seedlings. In this plantation 2–0 trees were inferior to both of the other classes of stock.

Success in planting in the region as a whole is very largely dependent upon adequate rainfall, especially during the first year or two after planting, and this was shown in the experiments made in the present study. The nearest weather station is at Wallace, Idaho, about 3 miles away and several hundred feet lower in elevation. The Weather Bureau records there indicated more than the average amount of rainfall in July and August for 1915 and 1916, the first two years after planting. Had this planting been done in the spring of 1917 or at any time during the next four years the results might have been quite different, for these were years of severe summer drought. Experiments in which the outcome is judged mainly by relative survival are likely to fail in such dry years, unless placed on the very best sites, because of universally high mortality. In like manner it has been observed that such experiments in moist years are often not very instructive for the opposite reason—mortality is so universally low that clear distinctions can not be made, except perhaps on the driest sites. Unfortunately, so far as the forests are concerned, drought can neither be controlled nor its occurrence predicted. Knowledge of drought hardiness must be accumulated year by year in the course of various observations on forest planting material.

HEIGHT GROWTH

Figure 1 shows the average elongation of stems or height growth of terminal shoots during the period of study.

It may be deduced from these measurements that the older the plant the greater is the height growth of stem. In general, the

growth of the 3-year-old trees (2–1 and 1–2 stock) was intermediate between the younger (1–1 and 2–0) and the older (2–2) trees each year. This relationship is especially noticeable in the cumulative effect of three years' growth. That the older plants grew faster than the younger is shown not only by a comparison of growth during any one year, but also by the accelerated growth of any one class of stock during the three years. Acceleration of height growth over a period of years is a matter of common observation on somewhat older seedlings.

It appears from Figure 1 that on all aspects the height growth of 2–0 stock in any given season was less than that of 1–2 stock. However, considering the fact that the transplanted stock was 1 year older than the seedlings, might it not be fairer to contrast the growth of 1–2 stock the first and second seasons after planting with that of 2–0 stock during the second and third seasons? An objection may be made that the seedlings had more time than the transplants to

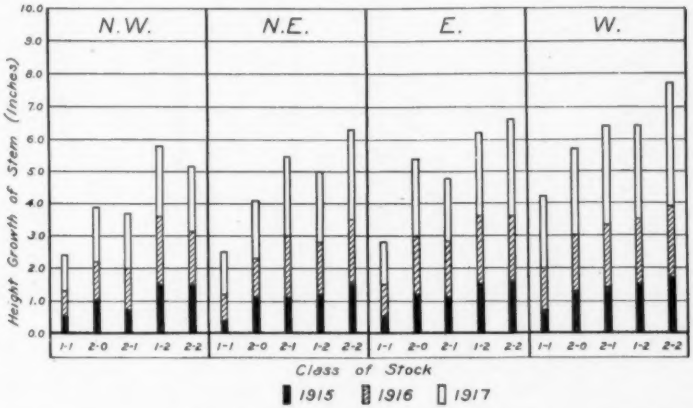

FIG. 1.—Height growth of stems of western white pine planted in the spring of 1915 on the Placer Creek area, Wallace, Idaho

recover from the shock of planting, but this objection would hardly apply to both the second and third years. Also, as stem growth occurs during the spring and early summer, it should not have been greatly influenced in this case by summer drought, especially as the months in question were above normal in amount of precipitation.

If this standard of comparison be accepted, and the growth data be charted as in Figure 2, it is evident that the 2–0 stock is growing at rates slightly faster on an average than those of the 1–2 stock. Also, by the same standard 1–2 stock is growing relatively faster than 2–2 on all aspects, making the slightly higher survival of the latter seem of less consequence. The significance of this comparison will be more apparent when relative planting costs are discussed.

Aspect also had a pronounced effect on growth, and the same trends are shown during each season and by each class of stock. The fastest stem growth took place on the west slope, then on the east, northeast, and northwest slopes in decreasing order. The

average growth of the planted stock during the 3-year period following planting was nearly 2 inches greater on the west slope than on the northwest, whereas the east and northeast slopes led the northwest by an inch and a half inch, respectively. Thus it may be seen that the poorest survival was accompanied by the most rapid growth, and vice versa.

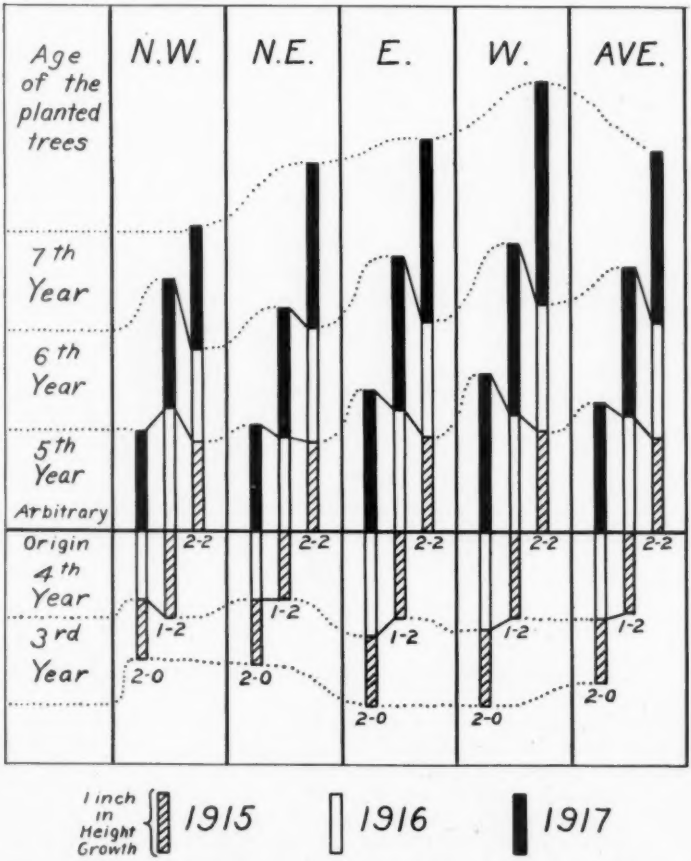

FIG. 2.—Comparison of growth of trees of same age. Stem height growth of different classes of stock during the first three years after planting

A somewhat obvious explanation of this opposite action on survival and on growth proves fallacious on analysis. Because considerable variation in development exists between individual plants of any class of stock, it seems at first glance reasonable to suppose that on drier sites only the superior plants survive, and consequently raise the growth averages for these sites above those taken on moist sites, where natural selection is less severe. In other words, it might be

reasoned that, when all survivors are considered, the quality of plants left after natural selection would be higher on the drier than on the moister sites, and that the difference might well be sufficient to account for the greater growth on drier sites.

This supposition will not "hold water," however, when a very simple test is applied. Inasmuch as survival of the different classes of stock on the moist northwest slope was fairly uniform and a wide divergence in the survival of these classes appeared on the dry west slope, it should follow that the stock on the west slope that survived least well, namely, the 2–0 and 1–1 lots, would show the greatest relative advance in height growth over that on the northwest aspect, for with them selection was most severe. However, when the decrease in survival for each class on the west slope below that on the northwest slope is expressed in percentages and compared directly with the percentage increase in growth on the west over the northwest, no consistent relation obtains. This is shown clearly in Table 2. Furthermore, the rather slight differences in survival on the northwest, northeast, and east slopes are in contrast to striking differences in growth. The hypothesis that natural selection following planting determines the growth rates on these sites does not seem tenable.

TABLE 2.—*Comparison of growth and mortality relations on northwest and west aspects three years after planting*

Age classes	Decrease in survival percentage on west aspect below that on northwest aspect	Per cent increase in growth on west aspect over that on northwest aspect
2–2	11.7	48.1
1–2	18.1	27.6
2–1	18.4	73.0
2–0	25.2	41.0
1–1	30.4	75.0

In seeking a truer explanation, a little speculation may be justified. In the complex interaction of environmental influences it may well be that light has a dominant rôle in the determination of the rate of growth on these slopes. The northwest slope receives but little more than diffuse light, whereas the west slope is exposed to direct sunlight in summer during the greater part of the day. Although little or nothing is known as yet of the optimum light intensity for synthesis of plant food—for assimilation, for cell division, and stem elongation of western white pine during its early development—it is possible to estimate from the figures for mortality from drought that transpiration, as well as evaporation from the soil, was greatest on the west aspect and least on the northwest. If, as is sometimes held, faster transpiration is accompanied by faster absorption of nutrient salts, sunlight may have been a controlling influence in the growth differences observed.

COST

Survival and growth have been considered in an effort to judge the relative merits of different age classes of planting stock and different aspects for planting sites. The cost per thousand of established trees is a better criterion where it can readily be determined. Cost is a factor that can never be disregarded in practice.

In comparing planting stock on the basis of survival costs it must be remembered that only relative differences for single years are comparable, because the cost of production and planting of tree stock fluctuates greatly from year to year with the price of labor and supplies. The same relative differences in cost should be fairly constant from year to year, however. The nursery costs shown in Table 3 are based on nursery practice and labor conditions existing in the spring of 1918 and include all expenses from seed collection to loading tree shipments on the train. The field cost is the same for all age classes and is taken from the cost of planting 1–2 white pine on the St. Joe National Forest in the spring of 1917. It includes shipping charges for nearly the same distance as for the stock used on the Wallace area. An obvious imperfection in this method of figuring lies in the fact that the smaller-sized trees can be both shipped and planted somewhat more cheaply than the larger. By neglecting these things a slight burden will be placed on the 2–0 and 1–1 stock and a corresponding advantage given to the 2–2 stock. The cost of survival may be computed by the following formula:

$$\text{Cost per 1,000 survivors} = 100 \times \frac{\text{total cost of planting 1,000 trees}}{\text{percentage of survival}}$$

Substitution in this formula of figures given in Tables 1 and 3 gives the data for Table 4.

TABLE 3.—*Costs of planting per 1,000 trees*

Age class	Nursery	Field	Total
2–2	$3.64	$9.99	$13.63
1–2	3.28	9.99	13.27
2–1	3.06	9.99	13.05
2–0	1.67	9.99	11.66
1–1	2.70	9.99	12.69

The data in Table 4 indicate clearly that for an equal investment in planting work more living trees were obtained with 2–0 stock than with any other age class tested when planting was done on favorable aspects such as the northwest, northeast, and east, and this advantage would have been accentuated had it been possible to consider in the figures the somewhat cheaper shipping and planting charges for this class of stock. On the severe west aspect the oldest stock, the 2–2, appears to have been less expensive, although when allowance is made for its having been perhaps unduly favored in the cost figures, the margin between the 2–2 and the 1–2 classes of stock may be considerably reduced. In general, 2–0 was least expensive, 1–1 most expensive, and the three older transplant classes were intermediate and a great deal alike. The universally high cost on the west aspect is due, of course, to the high mortality on that exposure.

TABLE 4.—*Cost per thousand trees surviving three years after planting on different aspects and with different age classes*

Aspect	Age classes					Average
	2-2	1-2	2-1	2-0	1-1	
Northwest	$13.75	$13.43	$13.62	$12.33	$13.57	$13.34
Northeast	13.97	13.40	13.49	12.71	14.42	13.60
East	13.88	13.49	13.61	11.96	14.40	13.47
West	15.59	16.44	16.86	16.80	20.11	17.16
Average	14.30	14.19	14.39	13.45	15.62	

SUMMARY

In general the survival of planted western white pine trees places 2–2 transplanted stock at the top, with 1–2, 2–1, 2–0, and 1–1 following in descending order. Height growth also conforms to this order. The cost of survival definitely favors 2–0 seedlings on moderate sites, and 2–2 or 1–2 only on the more severe sites.

Aspect of planting site is as important as the class of stock. Of the four exposures tested the lowest survival, accompanied by the fastest growth, occurred on the west exposure. The east, northeast, and northwest aspects showed considerably higher survival in the order given.

THE PERFECT STAGE OF THE FUNGUS WHICH CAUSES MELANOSE OF CITRUS[1]

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INTRODUCTION

A disease of citrus and related plants to which the common name melanose is applied was first recognized near Citra, Fla., by Swingle and Webber [2] in 1892. Their account of the disease, published in 1896, states that in their opinion it was caused by a "vegetable parasite" which they were not able to isolate in culture. In 1912 a paper by Fawcett [3] was published in which he set forth the results of his investigations on a type of stem-end decay of fruits, and he ascribed the cause of the decay to a previously undescribed organism which he designated *Phomopsis citri*. The relationship between this stem-end rot and melanose was not suspected at first. Evidence has been presented by Floyd and Stevens,[4] however, and by others who have investigated this problem, which shows that the two forms are undoubtedly caused by one and the same fungus. The rules of proof to establish this relationship have never been completely followed, because thus far it has not been possible for anyone to isolate *Phomopsis citri* from melanose lesions on leaves, twigs, and fruits.

In July, 1925, the present writer found, on fallen decaying twigs of lime (*Citrus aurantifolia* Swingle), on the grounds of the United States Citrus-Disease Field Laboratory, Orlando, Fla., a species of Diaporthe. Since several species of the form genus Phomopsis are known to have an ascigerous stage belonging to the genus Diaporthe, it was suspected that these specimens were those of the perfect stage of *Phomopsis citri*. Subsequent collections of the perfect stage were made in August and September from twigs of grapefruit (*Citrus grandis* Osbeck), sweet orange (*Citrus sinensis* Osbeck), and tangerine (*Citrus nobilis* Lour.), in the vicinity of Plymouth, Winter Park, and Gotha, Fla. A bundle of affected twigs bearing the Phomopsis stage was collected in September and placed in a sheltered location in contact with the ground. It was examined from time to time to watch the development of the perfect stage. During the last week in the following January it was found that perithecia had developed to maturity on the twigs.

These field observations when taken collectively indicate that the Diaporthe stage may be found at any time throughout the year on fallen citrus twigs.

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[1] Received for publication March 2, 1926; issued October, 1926.

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[4] FLOYD, B. F., and STEVENS, H. E. MELANOSE AND STEM-END ROT. Fla. Agr. Expt. Sta. Bul. 111: 5-16, illus. 1912.

ISOLATIONS

Two methods were employed in isolating this Diaporthe. Perithecial stromata were crushed in drops of sterile water on a slide, and loopfuls of the suspension were spread over the surface of hardened agar plates. The resultant growth consisted of colonies of Diaporthe and several other fungi. Transfers of selected colonies were made to potato-dextrose agar slants and several strains of Diaporthe in pure culture were obtained by this means. Much more satisfactory results, however, followed the inversion of hardened agar plates over twigs placed on strips of moistened blotter in the tops of Petri dishes. The ascospores were forcibly ejected and could be seen to have lodged on the surface of the agar, when examined under the low power of the microscope. The position of individual ascospores was marked on the bottom of the plates and their development noted at intervals. By the use of these inverted plates contaminations were avoided and pure cultures were readily obtained. Several cultures of the fungus were isolated by this method, in August and September, from collections of this Diaporthe stage on twigs of grapefruit, sweet orange, and tangerine. The mycelial growth of these several cultures on potato-dextrose agar slants resembled that of stock cultures of *Phomopsis citri* isolated from fruit affected with stem-end rot. Since Phomopsis forms pycnidia sparingly or not at all on this substratum, subcultures were made on sterilized stems of pigeon pea (*Cajanus indicus* Spreng). In three to four weeks pycnidial production occurred in abundance on this substratum. These pycnidia were of the Phomopsis type and bore both functional conidia (fig. 1, B) and stylospores (fig. 1, C). Comparative measurements were made of pycnidia, conidia and stylospores of *Phomopsis citri* from cultures isolated from stem-end rotted fruits, and of similar structures in the cultures from ascospores, and they were found to be similar in every respect.

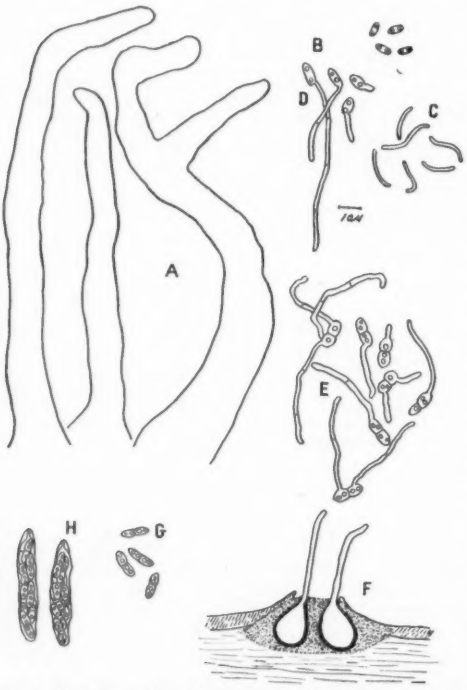

FIG. 1.—A, diagram shows shape of perithecial breaks; B, conidia of *Phomopsis citri* from ascosporic cultures; C, stylospores of *P. citri* from cultures from ascospores; D, germinating conidia of Phomopsis stage; E, germinating ascospores of *Diaporthe citri*, after growth for 16 hours; F, diagram of stroma and perithecia of *D. citri*; G, ascospores of *D. citri*; H, asci of *D. citri*

All cultures, except two which were obtained from ascospores, developed the conidial stage. These two, one of which was cultured on potato-dextrose agar and the other on pigeon-pea stems, were maintained under identical conditions and they produced perithecia (fig. 2). However, subcultures from these in turn developed the pycnidial stage. No adequate explanation can be given at this time to account for the production of perithecia by these two isolations. This problem is especially puzzling because thousands of isolations of Phomopsis from decaying fruit have been made during the last few years in the routine investigations in this laboratory and none has been found to develop perithecia.

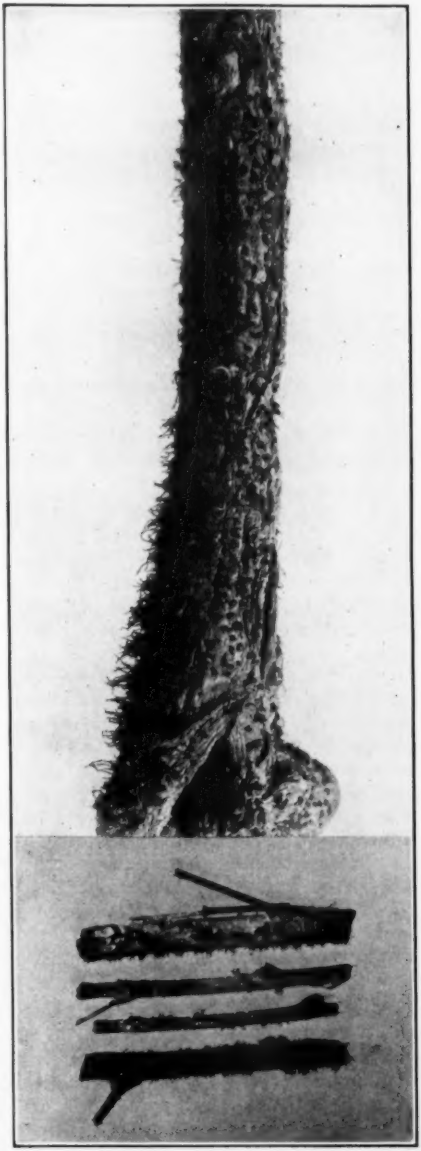

FIG. 2.—Perithecia of *Diaporthe citri* on grapefruit twig (above); and in culture on pigeon-pea stems (below)

THE ASCIGEROUS STAGE

The perithecia of this Diaporthe can be distinguished from most other fungi by the presence of slender black beaks which protrude singly or in small groups from the cortex of the twigs (fig. 2). A somewhat similar and related organism, *Eutypella citricola* Speg., which appears to be quite widely prevalent in Florida, also has these characteristics and may be confused with Diaporthe if one depends entirely upon examination with the unaided eye. Such at least was the writer's experience with collections made at De Land, Fort Pierce, Haines City, Winter Haven, Bartow, Fort Myers, Wauchula, Lakeland, and Sarasota, Fla., near all of which places melanose is prevalent. Further chance for confusion arises from the fact that Eutypella may occur interspersed with the citrus Diaporthe.

The perithecial stromata of Diaporthe occur within the bark and remain covered at maturity. The beaks vary in length from 200 to 800 μ, and in diameter from 40 to 60 μ (fig. 1, A). They are bluntly rounded at the apex and are entirely black, except the extreme tip, which is dark brown. The perithecia range from 125 to 160 μ in diameter and are embedded in a black stroma (fig. 1, F). The asci are sessile, elongate-clavate, 50 to 55 μ by 9 to 10 μ. They are thickened at the apex (fig. 1, H), and open by an apical pore. The ascospores are two-celled, slightly constricted at the septum, and each cell typically contains two prominent oil globules (fig. 1, G). They are hyaline, elongate-elliptical to spindle-shaped, and vary from 11.5 to 14.2 μ by 3.2 to 4.5 μ.

The ascospores germinate readily in tap water and on potato-dextrose agar. There is first a considerable increase in volume of the spore, which is followed by the emergence of a single germ tube or one from each cell (fig. 1, E). The mycelium soon becomes septate, and after 48 to 72 hours' growth the colonies are evident to the unaided eye.

INOCULATIONS

Because of the results of the comparative cultural studies, inoculations were attempted with cultures which were bearing pycnidia and which had been isolated from ascospores as the source of inoculum. Swabs of cotton were dipped in conidial suspensions and placed on tender leaves and twigs. These were then protected against desiccation by being wrapped for 24 to 36 hours in waxed paper, and after this time the wrappings were removed. Grapefruit and sweet-orange seedlings which were free from disease were employed in two of the tests. Young flushes of growth on large grapefruit trees were employed in another trial. Infections were evident in about a week, and within 12 to 14 days typical melanose lesions had developed on all inoculated parts. Near-by seedlings which remained free from melanose and uninoculated flushes which remained free served as controls.

Cultures from ascospores were used also to inoculate mature oranges. The surfaces of the fruits were first disinfected by washing with a 1:1000 solution of bichloride of mercury. The inoculum was then introduced through punctures in the sides, after which the fruits were placed in moist chambers. Fruit which was punctured but not inoculated served as controls. Several of the oranges, both inoculated and uninoculated, developed stem-end rot from natural infection around the stems. Infection developed around the punctures of all inoculated fruits, but not around any of those which were uninoculated.

TAXONOMY

The genetical relationship of the Diaporthe in question and *Phomopsis citri* is established both by the comparative cultural studies, as reported above, and by the pathogenicity of cultures from Diaporthe. Since this relationship is shown for the first time by these studies, and as the specific name *citri* was first employed for this organism, the name *Diaporthe citri* (Fawcett) n. n. becomes the appropriate binomial. Even though accounts by several other investigators contain the descriptive features of the Phomopsis stage, it appears best to summarize the characteristics of both stages of the fungus as follows:

Diaporthe citri (Fawcett) n. n.

Syn. *Phomopsis citri* Fawcett.

Ascogenous stage: Perithecia single or in groups, immersed in black stromata which are covered by the bark, 125 to 160 μ in diam.; beaks black, tapering, 200 to 800 μ in length; asci sessile, elongate-clavate, 50 to 55 μ by 9 to 10 μ, apex thickened and pierced by a narrow pore; ascospores hyaline, two-celled, constricted, elongate-elliptical to spindle-shaped possessing four guttulae, 11.5 to 14.2 μ by 3.2 to 4.5 μ.

Hab. Saprophytic on decaying twigs of *Citrus grandis*, *C. nobilis*, *C. sinensis* and *C. aurantifolia*.

Conidial stage: Pycnidia on dead branches and decaying fruit, scattered, dark, ovoid, thick-walled and parenchymatous, erumpent at maturity, 200 to 450 μ in diam.; conidia ovate to elongate, hyaline, guttulate, 5 to 9 μ by 2.5 to 4 μ, mostly 6 to 8 by 3 μ; stylospores slender, hooked, 20 to 30 μ by 0.75 to 1.5 μ.

Hab. Saprophytic or parasitic on leaves, stems, and fruits of Citrus and related plants. It attacks young leaves, stems, and fruits, causing melanosae; and on mature citrus fruits causes stem-end rot.

SUMMARY

The perfect stage of the organism which causes melanose and stem-end rot of citrus has been found to occur upon decaying twigs of lime, grapefruit, sweet orange, and tangerine.

Its connection with *Phomopsis citri* has been established by growth of both the pycnidial and ascogenous stages in culture from isolations from ascospores. Further, the conidia from cultures from ascospores have been employed successfully in producing melanose lesions on leaves of grapefruit and sweet orange and a rot of mature oranges.

The ascigerous stage belongs to the genus Diaporthe, and the binominal *Diaporthe citri* (Fawcett) n. n. is herein proposed.

THE RELATION OF STORAGE TO THE QUALITY OF SWEET POTATOES FOR CANNING PURPOSES [1]

By C. W. CULPEPPER and C. A. MAGOON, *Office of Horticulture, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

In an earlier paper (*10*) [2] the writers reported the results of studies on the sweet potato (*Ipomoea batatas*), dealing particularly with the canning quality of different varieties and giving some attention to methods of handling as affecting the quality of the canned product. Although the data presented therein dealt rather fully with the comparative merits of the different varieties when canned after the usual curing and storage period, that portion dealing with the significance of their behavior under differing conditions of treatment was suggestive rather than conclusive. The number of varieties studied in this connection was limited to four of the well-known sorts, and the chemical data based thereon were, accordingly, incomplete. The sweet potato is known to vary considerably in its composition, and with the results of but two seasons' work in hand, further information as to the constancy of the varietal differences noted would seem to be of considerable value.

In the use of canned sweet potatoes for culinary purposes it is sometimes desirable to have a product that is very firm and that will hold its form while being prepared for the table, as in the case of sautéing and candying. Some varieties which are attractive and satisfactory in other ways become soft when cooked, and are therefore unsuited for this purpose. A method of handling that would give this desired firmness in the product from the best table varieties would be of considerable advantage to the housewife.

In the earlier studies mentioned firmness or consistency tests were performed upon the product of all the varieties studied, which had been handled in the usual way, and like tests were made upon the product from a few of the varieties canned immediately after the potatoes were harvested. Among those of the first group a few were found which yielded a product of moderately firm consistency but one still too soft to be entirely satisfactory. The product from the freshly dug potatoes, on the other hand, was found to be very firm in all cases, and this fact seemed to suggest a possible solution of the problem.

It was for the purpose of investigating further this phase of sweet-potato utilization, and of making more complete the information available on the chemical constitution of the sweet potato and the transformations taking place within it under different conditions, that the work was pursued for another season. The results of this study are presented in the present paper.

The literature pertinent to this work has been reviewed already in the paper above mentioned, but reference will be made to it again

[1] Received for publication April 2, 1926; issued October, 1926.

[2] Reference is made by number (italic) to "Literature cited," p. 643.

from time to time in the present text wherever it will help to clarify the subject under discussion.

In all, 29 named varieties of sweet potatoes, together with about one-half dozen unnamed sorts were available for the work. These, arranged in alphabetical order, were: Ballinger's Pride, Big Stem Jersey, Catawba Yellow, Creola, Dahomey, Dooley, Florida, Georgia, Gold Skin, Gros Grandia, Haiti, Key West, Marneyita, Miles, Nancy Hall, Pierson, Porto Rico, Pumpkin "Yam," Purple "Yam," Red Bermuda, Red Brazil, Southern Queen, Triumph, Vineless Pumpkin "Yam," Vineless "Yam," White Sealy, Yellow Belmont, Yellow Jersey, and Yellow Strasburg. The unnamed sorts were identified by accession numbers only.

The potatoes were grown for the work at the Arlington Experiment Farm near Rosslyn, Va.

PLAN OF WORK

The plan of the experiments called for practical canning tests upon the potatoes immediately after they were dug and again after a month of storage at the end of the usual curing treatment,[3] particular attention being given to the relative firmness of the canned product, its appearance and table quality, and its adaptability to various culinary uses as determined by practical kitchen tests. Samples for chemical analysis were taken of each variety, both raw and after cooking, at the time the canning tests were made, in order that the causes for change in the physical characteristics of the canned product as the result of curing and storage might be carefully studied. The record of these experiments follows.

EXPERIMENTAL METHODS

The digging of the crop was started October 17, 1922, and the work of canning and sampling for chemical analysis was begun the same day. One lot of each variety was canned immediately and the other was placed in the curing chamber. As soon as the different varieties were canned the curing of the second lot was begun. The curing treatment was continued until November 3. On this date the fire was extinguished and the temperature allowed to drop to 55° to 65° F., at which it was maintained throughout the storage period. On November 27 the canning and sampling for chemical analysis was begun again and the final work completed on December 2, 1922.

Uniform canning methods were followed throughout the experimental work, and the technic of chemical sampling and analysis was the same as that used in previous studies (*10*).

The quantity of raw stock used in the canning experiments varied somewhat with the different varieties, but averaged about 30 pounds for the freshly dug potatoes and 25 pounds for the cured and stored stock. Where larger yields in the field permitted, the size of the sample was increased, and in some instances where yields were light and the amount of material available was less, the size of the sample was smaller.

[3] The curing treatment consisted in holding the potatoes in open slatted crates for 10 days in a curing chamber the temperature of which was maintained at about 85° F., with ample ventilation to allow the moisture driven off in the curing process to escape. At the end of this time the temperature was allowed to drop to 55° to 65° F. and the potatoes held under these conditions for one month.

Two types of canned product were packed: One, as whole potatoes, for which the No. 3 plain tin can was used; and the other, in the form of pulp or pie stock, for which No. 2 cans were employed.

Preparation for the can consisted in steaming the washed potatoes in a steam box until practically cooked (which required from 40 to 50 minutes) peeling at once by hand, packing hot into the cans, and sealing immediately. For the preparation of pie stock the hot freshly peeled potatoes were passed through the food grinder, placed immediately in the cans, and sealed.

TABLE 1.—*Peeling quality and percentage of waste of sweet potatoes canned fresh from the field and those canned after curing and storage*

Variety of sweet potato used	Sweet potatoes freshly dug			Sweet potatoes after curing and storage		
	Weight of sample in pounds	Peeling quality	Percentage waste	Weight of sample in pounds	Peeling quality	Percentage waste
Ballinger's Pride	40	Poor	12½	25	Poor to fair	17
Big Stem Jersey	30	Fair	13⅓	25	Poor	16
Catawba Yellow	30	do	16⅔	25	Fair to poor	18
Creola	8	do	18¾	7	Good	25
Dahomey	30	Poor	14	25	Poor	15
Dooley	30	Good	12½			
Florida	25	Fairly good	16	25	Poor	18
Georgia	30	do	14	25	Good	15
Gold Skin	30	do	11	25	do	16
Gros Grandia	8	Poor	18¾			
Haiti	30	do	11⅔	25	Fair	22
Key West	30	Very good	13⅓	25	do	23
Marneyita	12	Good	12½			
Miles	30	Poor	11⅔	25	Fair to poor	15
Nancy Hall	30	Fair	14	25	Good	15
Pierson	30	do	11	25	Poor	16
Porto Rico	60	do	13¾	25	Very good	16
Pumpkin "Yam"	30	Very good	15	25	Fair	15
Purple "Yam"	30	do	10	25	do	16
Red Bermuda	30	Poor	12½	25	do	14
Red Brazil	30	do	12½	20	do	16¼
Southern Queen	30	do	11	25	Good	14
Triumph	30	do	14	25	do	18
Vineless Pumpkin "Yam"	30	Good	11	16	do	17
Vineless "Yam"	18	Fair	14	10	Fair	17½
White Sealy	9	Very good	11	25	Poor	20
Yellow Belmont	35	Good	13½			
Yellow Jersey	30	Fair	13⅓	25	Good	13
Yellow Strasburg	30	Good	11⅔	25	Poor	34
No. 10412	30	Poor	11⅔	25	do	21
No. 10650	30	do	11⅔	25	Fair	28
No. 11284	30	do	11⅔			
No. 12686	30	Fair	13⅓	25	Good	14
No. 24013	30	Poor	11⅔	12	do	14½
No. 24014	30	do	13⅓	25	do	17
No. 24015	35	do	13½	10		27½

The processing was performed as quickly as a sufficient number of cans could be packed to fill the retort. While awaiting the processing, all cans were kept submerged in a tank of water held at about 80° C. to prevent the contents from cooling. No. 3 cans were processed in the steam retort for 1 hour at 116° C., and the No. 2 cans for 45 minutes at the same temperature.

Records were kept of the weight of each sample, the peeling quality, and the amount of waste of each variety as it was handled.

At the end of the canning season samples of the different products from each of the varieties were opened for critical study, and their comparative qualities judged by a committee made up of canned-food experts, specialists in home economics, and others qualified by

experience to judge the merits of the products. Other series of samples from each of the varieties were subjected to plasticity tests to determine the relative firmness of the products, and products from selected representative varieties were turned over to the experimental kitchen of the Bureau of Chemistry for practical cooking tests.[4]

TESTS FOR PEELING QUALITY AND WASTE

Table 1, which records the findings made at the time the canning was done, tells its own story and calls for little comment. Owing to the fact that the bulk of the material used in each case was small, the figures can not with safety be used as an index of just what percentage loss would obtain when large-scale canning operations are carried on. When analyzed in connection with the previous work (*10*), however, they give a fair idea of what may be expected under commercial conditions.

It is seen that in the case of both the freshly dug stock and that which had been cured and stored, the peeling quality ranged from poor to very good. In the case of about a dozen different varieties the peeling quality was satisfactory under both conditions, while half that number peeled better in the freshly dug condition, and about a dozen better after the curing and storage.

The percentage of waste ranged from 10 to 18¾ per cent in the freshly dug stock and from 13 to 34 per cent in the cured and stored material. Variation in the amount of waste in the freshly dug potatoes was due primarily to the size of the potatoes; but in the cured stock some loss was sustained through discoloration caused by fungi which occasionally affected individual potatoes.

PLASTICITY TESTS

Of particular interest in the present study was the relative firmness or plasticity of the different products from the same varieties when canned fresh from the field as compared with those from cured and stored stocks. Plasticity tests, the results of which are given in Table 2, were made by observing the resistance to penetration of the canned material by a plunger having a cross-section of one-half square centimeter. The figures given represent the number of grams pressure required to force the plunger into the product to a distance of 2 cm. in 1 minute of time, and are averages from several tests made upon the material at both ends of the can.

The varieties are arranged in the order of greatest resistance in the material from freshly dug potatoes.

The outstanding fact revealed by these figures is the very great difference in plasticity of the product from the freshly dug potatoes as compared with that from the cured stock. The pressure required to force the plunger into the material in the first series ranged from 250 to 1,400 gm., whereas in the product from the cured potatoes only 20 to 180 gm. of pressure was required. In other words, the softest product from the freshly dug potatoes was considerably firmer than that of the driest material obtained after the storage treatment. In every instance there was a very marked reduction in the firmness as a result of the storage.

[4] The writers are indebted to L. H. Bailey, of the Bureau of Chemistry, who superintended this part of the work, to Miss R. L. Rutledge, who made the cooking tests, and to F. C. Blanck, W. W. Skinner, P. L. Gowen, and H. C. Gore, also of the Bureau of Chemistry, who passed judgment upon the various sweet-potato dishes prepared from these products.

TABLE 2.—*Relative plasticity of the products from sweet potatoes of different varieties when canned fresh from the field and after curing and storage*

[The figures represent the pressure in grams required to force the plunger into the material to a distance of 2 cm. in 1 minute of time]

Variety of sweet potato used	Resistance of sweet potato when—		Variety of sweet potato used	Resistance of sweet potato when—	
	Freshly dug	Cured and stored		Freshly dug	Cured and stored
	Grams	*Grams*		*Grams*	*Grams*
Yellow Belmont	1,400		Marneyita	575	
Florida	1,000	65	Nancy Hall	550	20
Purple "Yam"	975	180	No. 24013	550	110
No. 24015	950	80	Yellow Strasburg	525	100
Gros Grandia	950		No. 12686	500	70
Vineless "Yam"	950	50	Ballinger's Pride	500	40
No. 24014	875	80	Dooley	500	
White Sealy	750	55	No. 10412	475	120
Pierson	725	100	No. 10650	450	75
Vineless Pumpkin "Yam"	700	30	Gold Skin	425	80
Dahomey	675	180	Red Bermuda	425	80
Creola	650	25	Key West	375	70
No. 11284	625		Yellow Jersey	375	180
Haiti	625	85	Big Stem Jersey	350	120
Miles	600	40	Catawba Yellow	300	35
Pumpkin "Yam"	600	40	Red Brazil	275	85
Triumph	600	85	Southern Queen	275	60
Georgia	575	45	Porto Rico	250	25

It is noted that there are relatively few varieties showing any marked degree of firmness in the product from the stored potatoes. Two of these, Dahomey and Purple "Yam," because of their elongated rootlike form, rather poor peeling quality, and tendency to discolor in the can are hardly suited for general canning purposes. The two Jersey potatoes, Big Stem Jersey and Yellow Jersey, because of their favorable shape and size and their desirable table qualities are used more extensively for canning than any other of the drier sorts. It is seen, however, that where firmness combined with high color is desired in the finished product a wide range of varieties is available if the potatoes are canned when freshly dug.[5]

That a better idea may be gained of the difference in firmness or plasticity of the sweet-potato product when it is canned fresh from the field and after it has been cured and stored for one month, Figures 1 to 4, inclusive, are shown. The variety used in the illustrations was Nancy Hall, well known as one of the best soft cooking varieties. Figure 1 shows the product from the freshly dug potatoes canned whole. Figure 2 shows the same, canned after the curing and storage treatment. Figures 3 and 4 illustrate the product canned as pie stock under the same conditions as in Figures 1 and 2.

Little comment on these illustrations is required. Attention is called to the firmness of the products shown in Figures 1 and 3 as compared with the softness and moistness of the products illustrated in Figures 2 and 4.

It will be of interest at this point to consider the chemical composition of the different varieties of sweet potatoes in relation to the difference in plasticity, as the changes in physical characteristics are due to alterations in the chemical constitution of the potatoes as a result of the curing and storage treatment.

[5] Those interested in the color factor in the canned product of sweet potatoes will find a series of color plates showing the range of colors found among the different varieties, in the U. S. Department of Agriculture Bulletin No. 1041 entitled, "A Study of Sweet-Potato Varieties, with Special Reference to Their Canning Quality" (*10*).

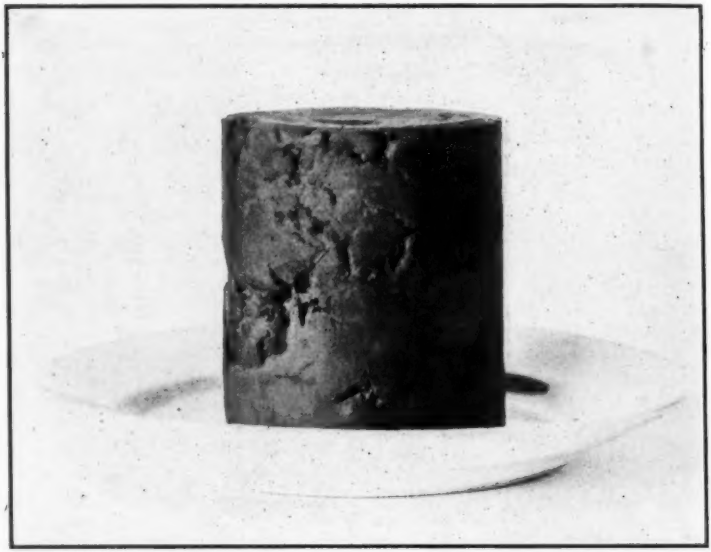

FIG. 1.—Product of the Nancy Hall sweet potato canned whole immediately after digging

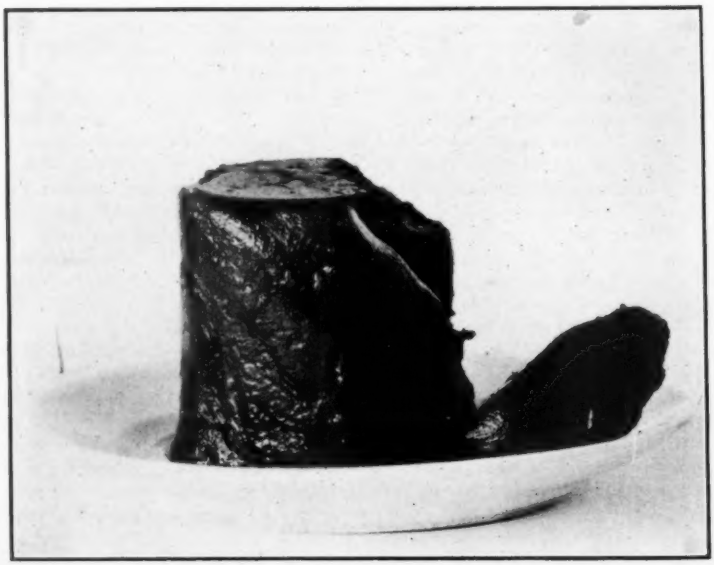

FIG. 2.—Product of the Nancy Hall sweet potato canned whole after the curing and storage treatment

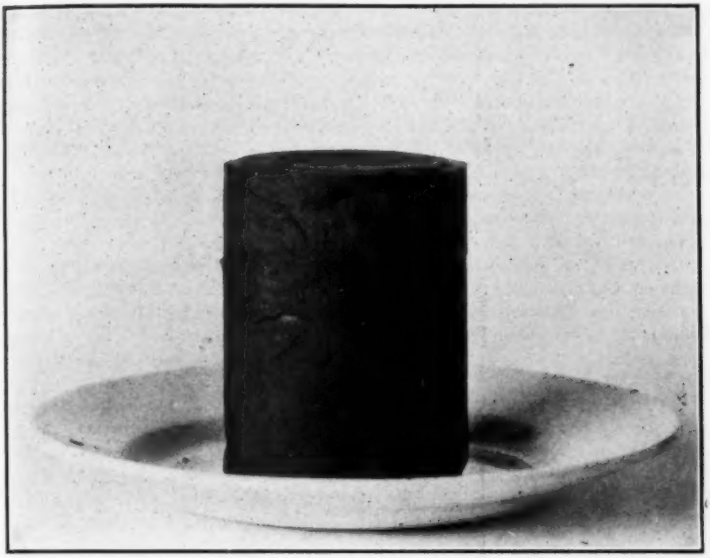

FIG. 3.—Product of the Nancy Hall sweet potato canned as pie stock immediately after digging

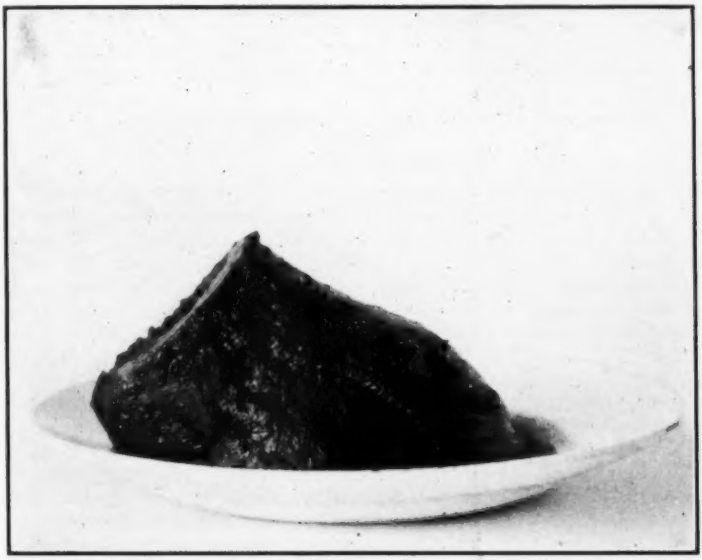

FIG. 4.—Product of the Nancy Hall sweet potato canned as pie stock after the curing and storage treatment

CHEMICAL STUDIES

These studies are a continuation and extension of chemical work previously reported by the writers (*10, 11*), and is here made to include all the important varieties of sweet potatoes grown in this country at the present time. The methods of analysis were essentially the same as reported in the earlier work. Samples were taken from a number of representative potatoes both raw and cooked, at the time of digging and after curing and storage.

To 100 gm. of the sample enough 95 per cent alcohol was added to make the alcohol concentration 75 to 80 per cent. After extraction with alcohol the extract was made up to volume and the sugars determined in the usual way by the copper reduction method. The sucrose was inverted by boiling with 2 per cent citric acid, as recommended by Davis and Daish (*2*), in order to avoid inversion of maltose. The total polysaccharides were determined [6] by inversion with hydrochloric acid as recommended by the Association of Official Agricultural Chemists (*1*). The dextrin was estimated as described by the writers (*11*), except that the tedious filtrations were avoided by centrifuging at high speed in a large centrifuge of standard make.

The reducing sugars were calculated in two ways: In the first, the total reducing sugars both before and after cooking of the potatoes were calculated as invert sugar; in the second, the increase after cooking was calculated as maltose. In making this estimate it was assumed that all the sugar formed during cooking was maltose. This method of estimating maltose is, of course, indirect and is subject to the error of random sampling. Owing to the varying amounts of the different sugars in such a complex mixture the method may be subject to considerable error. In most cases the estimated maltose very nearly accounted for the decrease in starch during cooking.

The results of this work are presented in Table 3.

MOISTURE CONTENT

Table 3 shows that the samples vary considerably in their percentage of dry matter. Johnstone (*8*) found characteristic differences in sweet potatoes of the Porto Rico and Triumph varieties. Harrington (*5*) investigated the moisture content of sweet potatoes during storage and found a decrease from 71.34 to 63.5 per cent after several months of storage. His results showed that there was a considerable variation in the moisture content of potatoes of different varieties. Shiver (*13*) found that the moisture content was variable during storage, and the results of many other investigators indicate that the moisture varies with the seasonal conditions and with the methods of storage after digging.

The present results show a variation in the moisture content of from 27 to 39 per cent. In almost any lot individual potatoes of any variety will vary fron 4 to 6 per cent in moisture. Numerous determinations have shown that in general the larger the potatoes the higher the moisture content. However, the age of the potatoes is a factor, as has been shown by Keitt (*9*). He found that the young

[6] The determinations of total polysaccharides were made by B. C. Brunstetter.

potato had a high solid content followed by a period of rapid growth in which the solid content was low. As the potato approached maturity the growth was slower while starch was being laid down, resulting in a higher solid content in the mature roots.

Because of these variations in moisture content the sampling error may sometimes be considerable in spite of efforts to avoid it. In the present results there are a few cases in which the moisture of the raw and the cooked potatoes varied from 1 to 2 per cent. However, the differences are usually less than 1 per cent. In most cases where there are differences shown the raw sample is higher, though in several cases the reverse is true. Only a small amount of evaporation was possible in the method of handling used and the differences shown are mainly due to sampling error.

Generally, the moisture content found is characteristic of the variety, with the possible error of 2 or 3 per cent. Triumph, Georgia, and Purple "Yam" are characterized by a very high solids content. Numerous moisture determinations on these during the last four years have shown a solids content varying from 35 to 40 per cent, with an average of about 37 per cent. Gold Skin has the lowest solids content of any variety, and though this result is somewhat below the average for several years, it is always strikingly low in solids. This probably explains why potatoes of this variety are likely to shrivel and shrink severely under certain storage conditions. The Jersey group as a whole is below the average in solids. Key West and Creola are also low in solids, which partly accounts for their consistency when cooked. Porto Rico is also frequently rather low although the determination in this case is somewhat below the average for four years. Potatoes of a larger number of varieties are quite similar in their solids content and average from 32 to 35 per cent.

The quantity of moisture present is an important factor in causing the difference in quality of varieties by modifying the firmness or softness of the canned product. The very high solids content makes the sweet potato a very concentrated food.

SUGARS

Miyake (*12*) identified dextrose, levulose, and sucrose in the sweet potato. He obtained no evidence, however, of the presence of pentose, galactose, mannose, or maltose. A number of workers (*5*, *6*, *7*, *9*, *10*, *11*, *12*, *13*, *14*) have reported the presence of both reducing and nonreducing sugars in the sweet potato. The writers (*10*) found large quantities of reducing sugars in the cooked potatoes, and later showed (*11*) that large quantities of these sugars, principally maltose, were formed during cooking.

Gore (*4*) found that practically all the sugar formed during the cooking of sweet potatoes was maltose. He also found (*3*) that the sweet potato was rich in diastase, and that only a small quantity of malt was necessary to convert all the starch into sugar. Hasselbring and Hawkins (*6*, *7*) found that the sugar content of the sweet potato changed after digging and varied with the storage temperature. At low temperatures the sucrose increased and the reducing sugars decreased, which led them to the conclusion that starch was first changed to reducing sugars and that sucrose was then synthesized from these.

TABLE 3.—*Results of chemical analysis of sweet potatoes of different varieties*

Variety of sweet potato	Condition	Analysis made immediately after digging (per cent)												Analysis made after curing and storage (per cent)										
		Solids			Sugars							Polysaccharides		Solids			Sugars						Polysaccharides	
					Calculated as invert			Increase after cooking; calculated as maltose									Calculated as invert			Increase after cooking; calculated as maltose					
		Total	Alcoholic extract	Residue	Total	Reducing	Sucrose	Total	Reducing	Sucrose	Maltose	Total	Dextrin	Total	Alcoholic extract	Residue	Total	Reducing	Sucrose	Total	Reducing	Sucrose	Maltose	Total	Dextrin
Ballinger's Pride	Raw	31.22	3.22	28.00	2.04	0.31	1.73					22.80		33.36	7.79	25.57	5.85	0.23	5.62					20.45	
	Cooked	32.52	14.20	18.32	9.88	8.00	1.88	12.62	0.31	1.88	10.43	13.21	0.08	32.82	19.94	12.88	13.62	8.12	5.50	18.34	0.23	5.50	12.61	10.50	1.82
Big Stem Jersey	Raw	32.26	4.52	27.74	2.96	.48	2.48					21.59		29.68	7.38	22.30	6.14	.83	5.31					17.84	
	Cooked	32.55	14.26	18.29	8.68	6.45	2.23	11.35	.48	2.23	8.64	14.13	.51	29.97	15.00	14.97	10.62	5.27	5.35	12.02	.83	5.35	5.84	11.97	1.24
Catawba Yellow	Raw	34.92	4.24	30.68	2.05	.78	1.27					24.84		33.28	7.62	25.66	6.17	.56	5.61					20.52	
	Cooked	34.92	15.68	19.24	9.14	8.01	1.13	12.72	.78	1.13	10.81	15.19	1.05	34.48	20.60	13.88	13.64	8.18	5.46	17.60	.56	5.46	11.58	10.10	5.09
Creola	Raw	29.11	3.12	25.99	1.41	.61	.80					20.77		31.16	8.38	22.78	6.45	1.07	5.38					18.22	
	Cooked	30.25	14.40	15.85	8.16	6.76	1.40	10.83	.61	1.40	8.82	12.68	.40	31.42	19.76	11.66	13.57	7.84	5.73	16.76	1.07	5.73	9.96	9.12	6.13
Dahomey	Raw	33.11	4.20	28.91	2.88	.52	2.36					25.12		33.66	7.60	26.06	6.22	1.37	4.85					20.84	
	Cooked	33.00	13.89	19.11	9.24	6.94	2.30	12.16	.52	2.30	9.34	15.28	.36	34.17	17.20	16.97	11.37	6.91	4.46	13.61	1.37	4.46	7.78	13.57	.62
Dooley	Raw	32.73	3.32	29.41	1.68	.18	1.50					23.52		35.85	8.13	27.72	5.87	1.39	4.48					22.17	
	Cooked	34.81	16.42	18.39	9.29	7.65	1.64	12.96	.18	1.64	11.14	14.71	.85	36.11	22.23	13.88	14.32	9.37	4.95	18.30	1.39	4.95	11.96	11.10	6.64
Florida	Raw	35.81	3.18	32.63	1.85	.15	1.70					26.10		36.57	7.66	28.91	5.57	.34	5.23					23.22	
	Cooked	36.18	17.16	19.02	9.91	7.70	2.21	14.02	.15	2.21	11.66	15.22	.00	36.62	20.52	16.10	13.78	8.12	5.66	17.82	.34	5.66	11.82	12.68	.06
Georgia	Raw	38.29	3.96	34.33	1.96	.21	1.75					27.46		37.27	9.58	27.69	7.81	.96	6.85					22.15	
	Cooked	37.83	15.20	22.63	9.19	7.62	1.57	12.80	.21	1.57	11.02	17.10	.81	38.25	23.68	14.57	16.28	8.94	7.34	20.25	.96	7.34	11.95	11.64	3.16
Gold Skin	Raw	28.58	5.30	23.28	4.03	1.02	3.01					18.42		29.06	10.08	18.98	8.09	1.95	6.14					15.18	
	Cooked	27.87	13.48	14.39	8.92	5.68	3.24	10.40	1.02	3.24	6.14	11.61	.73	30.63	17.40	13.23	12.54	6.14	6.40	13.67	1.95	6.40	5.32	10.58	1.32
Haiti	Raw	34.61	4.32	30.29	2.89	.75	2.14					24.23		33.65	6.64	27.01	5.29	.14	4.15					21.61	
	Cooked	34.62	15.80	18.82	9.74	7.42	2.32	12.94	.75	2.32	9.87	15.05	.07	33.41	20.28	13.13	13.16	8.56	4.60	16.75	1.14	4.60	11.01	10.50	1.16
Key West	Raw	30.58	2.94	27.64	1.87	.81	1.06					22.11		32.43	6.63	25.80	5.08	.59	4.49					20.64	
	Cooked	30.97	14.80	16.17	8.46	7.14	1.32	11.29	.81	1.32	9.16	12.93	.77	33.01	19.07	13.94	13.45	8.81	4.64	15.87	.59	4.64	10.64	10.86	2.19
Marneyita	Raw	31.39	4.49	26.90	2.72	.37	2.35					21.52		34.61	7.14	27.47	5.78	.51	5.27					21.97	
	Cooked	32.49	13.72	18.77	8.70	6.54	2.16	11.42	.37	2.16	8.89	15.01	.01	34.78	23.27	11.51	15.24	9.88	5.36	19.88	.51	5.36	14.01	10.00	6.72
Miles	Raw	32.47	3.92	28.55	2.16	.35	1.81					25.98		32.72	7.76	24.96	5.80	.47	5.33					20.43	
	Cooked	35.54	16.38	19.16	10.01	8.04	1.97	13.90	.35	1.97	11.58	15.32	1.00	35.34	21.28	14.06	13.54	8.37	5.17	17.68	.47	5.17	12.04	10.86	4.37
Nancy Hall	Raw	36.56	3.66	32.90	1.89	.17	1.72					26.32		33.95	9.22	24.73	7.96	.79	7.17					19.96	
	Cooked	36.57	18.72	17.85	10.70	8.60	2.10	15.05	.17	2.10	12.78	14.28	1.03	35.73	24.96	10.77	16.64	9.59	7.05	20.86	.79	7.05	13.02	8.07	6.20

Pierson	Raw	35.00	3.54	31.46	2.59	.46	2.13					25.16		34.77	7.00	27.77	4.94	1.43	3.51					22.21	
	Cooked	34.23	16.40	17.83	9.66	7.60	2.06	13.08	.46	2.06	10.56	4.26	.01	34.96	18.40	16.56	11.93	7.96	3.97	14.96	1.43	3.97	9.56	13.25	.06
Porto Rico	Raw	30.16	4.92	25.24	3.29	.85	2.44					21.19		31.88	8.24	23.64	6.66	.94	5.72					18.91	
	Cooked	31.94	18.24	13.70	11.04	8.41	2.63	16.30	.85	2.63	12.82	10.26	1.32	32.87	23.04	9.83	15.75	9.76	5.99	20.57	.94	5.99	13.64	7.37	6.09
Pumpkin "Yam"	Raw	34.04	3.16	30.88	2.15	.15	2.00					24.70		35.65	11.10	24.55	9.47	1.10	8.37					19.64	
	Cooked	35.02	16.32	18.70	9.21	7.53	1.68	11.01	.15	1.68	9.18	14.96	.04	34.25	22.44	11.81	15.08	7.57	7.51	18.07	1.10	7.51	9.46	9.44	4.78
Purple "Yam"	Raw	37.73	4.84	32.89	2.32	.18	2.14					26.60		36.58	8.22	28.36	6.15	.29	5.86					22.68	
	Cooked	38.11	17.48	20.63	10.06	8.27	1.79	13.76	.18	1.79	11.79	16.50	(1)	38.08	20.80	17.28	14.11	8.09	6.02	18.63	.79	6.02	11.82	13.82	.01
Red Bermuda	Raw	33.90	4.51	29.39	2.61	.52	2.09					23.51		32.82	6.95	25.87	5.58	1.12	4.46					20.69	
	Cooked	32.14	16.84	15.30	9.46	7.49	1.97	12.69	.52	1.97	10.20	12.84	.05	33.77	20.68	13.09	13.54	9.04	4.50	17.56	1.12	4.50	11.94	10.47	1.84
Red Brazil	Raw	33.76	4.16	29.60	2.79	.58	2.21					24.25		31.60	6.04	25.56	4.46	.72	3.74					20.44	
	Cooked	34.98	17.96	17.02	10.73	8.66	2.07	14.65	.58	2.07	12.00	13.14	1.38	32.72	17.56	15.16	11.43	8.05	3.38	14.96	.72	3.38	10.86	11.85	1.83
Southern Queen	Raw	33.30	3.96	29.34	2.77	.51	2.26					23.97		32.69	7.60	25.09	5.73	.64	5.09					20.07	
	Cooked	31.60	15.28	16.32	9.09	7.01	2.08	12.09	.51	2.08	9.50	12.05	1.41	33.67	21.04	12.63	14.02	8.92	5.10	18.38	.64	5.10	12.64	10.10	4.66
Triumph	Raw	38.28	3.98	34.30	2.38	.28	2.10					27.94		38.02	7.60	30.42	6.04	.72	5.32					24.13	
	Cooked	38.29	19.04	19.25	11.27	9.48	1.79	16.30	.28	1.79	14.23	16.12	.98	37.84	22.84	15.00	15.53	9.76	5.77	20.51	.72	5.77	14.02	11.64	1.79
Vineless Pumpkin "Yam"	Raw	33.94	4.62	29.32	2.85	.38	2.47					23.87		34.64	8.50	26.14	6.82	.45	6.37					20.91	
	Cooked	34.39	16.24	18.15	9.78	7.56	2.22	13.18	.38	2.22	10.58	14.34	.67	36.17	22.64	13.53	15.19	8.46	6.73	19.24	.45	6.73	12.06	10.65	5.16
Vineless "Yam"	Raw	35.78	3.50	32.28	1.98	.17	1.81					29.82		36.79	7.84	28.95	6.46	.31	6.15					23.16	
	Cooked	36.42	15.01	21.41	9.03	7.31	1.72	12.35	.17	1.72	10.46	17.91	.00	36.55	21.68	14.87	14.75	7.74	7.01	18.42	.31	7.01	11.10	11.89	3.61
White Sealy	Raw	32.09	3.94	28.15	2.41	.46	1.95					22.92		34.78	7.24	27.54	5.31	1.00	4.31					22.03	
	Cooked	32.01	14.04	17.97	8.92	7.11	1.81	10.30	.46	1.81	8.03	14.37	.02	33.05	20.44	13.21	13.62	8.87	4.75	17.69	1.00	4.75	11.94	10.56	1.24
Yellow Jersey	Raw	29.17	5.02	24.15	3.14	.61	2.53					19.42		30.48	8.66	21.82	6.79	.91	5.88					17.55	
	Cooked	30.32	13.86	16.46	8.87	5.95	2.92	10.77	.61	2.92	7.24	13.00	.32	29.62	11.92	17.70	11.81	6.14	5.67	13.74	.91	5.67	7.16	10.05	1.46
Yellow Strasburg	Raw	33.39	3.96	29.43	2.76	.78	1.98					23.54		37.98	8.84	29.14	6.57	1.15	5.42					23.51	
	Cooked	34.01	16.72	17.29	9.90	7.78	2.12	13.72	.78	2.12	10.82	13.83	.90	38.65	23.04	15.61	15.77	9.76	6.01	20.37	1.15	6.01	13.21	12.18	1.13
No. 10412	Raw	32.62	4.28	28.34	2.36	.48	1.88					23.42		34.91	6.48	28.43	4.73	.61	4.12					23.52	
	Cooked	34.21	18.32	15.89	10.64	8.46	2.18	14.60	.48	2.18	11.94	12.21	.69	35.34	19.60	15.74	12.62	8.23	4.39	16.44	.61	4.39	11.44	13.29	1.99
No. 12086	Raw	34.86	4.47	30.39	3.06	.93	2.13					24.35		32.19	7.58	24.61	5.74	1.65	4.09					19.76	
	Cooked	34.42	17.68	16.74	10.23	8.25	1.98	13.75	.93	1.98	10.84	13.39	.17	32.89	19.56	13.33	13.47	8.74	4.73	16.76	1.65	4.73	10.38	10.66	1.93
No. 24013	Raw	34.42	3.42	31.00	1.62	.15	1.47					24.80		35.24	9.26	25.98	8.40	.41	7.99					20.48	
	Cooked	34.02	15.20	18.82	8.81	6.90	1.91	12.27	.15	1.91	10.21	14.65	.03	35.40	22.00	13.40	14.20	6.87	7.33	17.20	.41	7.33	9.46	10.91	1.28
No. 24014	Raw	30.96	3.76	27.20	1.85	.28	1.57					21.76		33.38	7.68	25.70	5.81	1.03	4.78					21.36	
	Cooked	33.90	14.60	19.30	8.82	6.97	1.85	11.62	.28	1.85	9.49	15.44	1.01	34.81	20.24	14.57	13.54	8.57	4.97	17.35	1.03	4.99	11.33	11.05	2.40
No. 24015	Raw	33.93	3.86	30.07	2.64	.77	1.87					24.05		35.55	11.22	24.33	9.06	.86	8.20					19.46	
	Cooked	33.91	13.08	20.83	8.80	6.71	2.09	11.39	.77	2.09	8.53	16.64	.00	36.86	22.02	14.84	14.86	6.92	7.94	17.51	.86	7.94	8.71	11.87	2.60

[1] Trace.

Reducing Sugars

Table 3 shows that the reducing sugars in the uncooked potatoes were always small in amount and quite variable in different samples, even in the same variety. Since the quantity present is nearly always less than 1 per cent these variations are not large in total amount and are of no particular significance in canning operations. In most cases there is an increase in the amount present during the curing and storage period. There are some cases, however, where the reverse is true.

Maltose

After cooking there is always a very great increase in the reducing sugars. If this increase is calculated as maltose there appears to be 7 to 14 per cent present in the different varieties. This increase is accompanied by a corresponding decrease in starch. This indicates that there is a large amount of diastase present which varies considerably in the different varieties. Since maltose arises from the conversion of starch this change greatly affects the softness of the canned product. However, the softness is not proportional to the amount of maltose present, especially in the different varieties. Inconsistencies such as this lead one to conclude that there may be other very important factors that are also unexplained.

Among those varieties high in maltose are Triumph, Nancy Hall, Porto Rico, Yellow Strasburg, Purple "Yam," Georgia, Miles, and Marneyita. Generally there is at least a small increase in the maltose in the cooked samples after curing and storage, though in some cases the reverse is true, which may be due merely to sampling error. The large quantity of maltose present greatly affects the quality of the product by its added sweetness; but because of its lesser sweetening power it is not so important as sucrose.

Sucrose

Sucrose is the most abundant sugar in the raw material and the variation in the amount of it present greatly affects the sweetness of the canned material. There seems to be practically no change in it during cooking. Such variations as occur in the analyses seem to have resulted from errors in sampling. An increase in sucrose always occurs during curing and storage. There is from three to four times as much present in the cured as in the freshly dug samples. This tremendously affects the sweetness and the quality of the canned material and is the chief factor giving higher quality to the cured and stored material. There is not a very great variation in the amount of sucrose in potatoes of different varieties when freshly dug, but the variations are greater in the cured material, ranging from 3.5 to 7.5 per cent. Nancy Hall, Pumpkin "Yam," Vineless Pumpkin "Yam," Vineless "Yam," Georgia, Yellow Strasburg, and Porto Rico are particularly high in sucrose in the cured and stored material.

POLYSACCHARIDES

Starch

The hydrolyzable polysaccharides of the raw sweet potato consist almost entirely of starch. The amount present differs considerably with the varieties, stage of growth, and storage conditions. In the raw state the percentage of starch present is correlated quite closely

with the moisture content, being high when the moisture is low. It decreases during storage as a result of its conversion into sucrose. During cooking large quantities are converted into soluble products, chiefly maltose, but in some varieties considerable quantities are changed to dextrinlike forms. Starch is the chief constituent giving firmness to the canned product. In most varieties there is more or less starch remaining which, during the cooking process, is modified only by being gelatinized. The consistency of the canned material is very nearly proportional to the ratio of starch to moisture. Some of the raw samples are high in starch, namely, Triumph, Florida, Catawba Yellow, Nancy Hall, Vineless "Yam," and, immediately after digging, Pumpkin "Yam."

Dextrin

Stone (*14*) very early found dextrin present in cooked sweet potatoes and showed that it was formed during cooking. The writers (*10*), working with four varieties, found comparatively wide variations in the dextrin content and showed that there was close correlation between the amount of dextrin present and the softness of the cooked product. The dextrin arises from the partial conversion of starch during cooking probably as the result of the activity of diastase or other enzymes.

By comparing the figures in Table 2 showing relative consistency with those for dextrin in Table 3, it will be noted that in every case where there is a high dextrin content the material is soft in consistency. High dextrin is nearly always associated with high maltose content, but in many cases there is a high maltose content unaccompanied by high dextrin content. Dextrin does not seem to affect the quality except as it does so through modifying the consistency.

QUALITY OF PRODUCTS

It is hardly possible to convey by the usual terms just what is meant by "good" or "poor" canning quality when speaking of freshly dug sweet potatoes. High quality in sweet potatoes is, as already noted, usually associated with high sugar content, pleasing flavor, attractive color, and proper consistency. In the product from the freshly dug potatoes the sucrose content is relatively low, the flavor is less well developed, and the consistency always firm. Quality in this case applies more particularly to attractiveness of the product as determined by color and brightness; somewhat less to flavor; but particularly to its suitability for certain culinary uses for which the cured potato, because of its soft consistency, is not adapted. This product would supplement rather than suplant the usual canned sweet potato.

In the present studies both the freshly dug and the cured potatoes were canned and the product of each subjected to careful judging as to quality. With respect to the product from the cured potatoes the findings were in all cases practically identical with those previously obtained, which have been set forth in more or less detail by the writers in the publication previously mentioned (*10*). There it is recorded that Gold Skin was given first place, and Porto Rico, Nancy Hall, and Vineless Pumpkin "Yam" of the moist fleshed group, and Big Stem Jersey, Improved Big Stem, Yellow Strasburg, and

Triumph of the firmer fleshed varieties were accorded high ratings in about the order given.

Observations upon the canning qualities of the freshly dug sweet potatoes of the different varieties in the present study are given briefly in Table 4.

TABLE 4.—*Qualities of the canned product from freshly dug sweet potatoes*

Variety of sweet potato used	Attractiveness	Consistency	Tendency to darken on exposure	Table quality
Ballinger's Pride	Fair to good	Firm	Slight	Poor.
Big Stem Jersey	do	Fairly firm	Little	Medium to poor.
Creola	do	Firm	do	Fair.
Dahomey	Poor	Very firm	Pronounced	Poor.
Dooley	Fair	Firm	Little	Fair.
Florida	do	Very firm	do	Poor.
Georgia	Poor	Firm	Pronounced	Do.
Gold Skin	Very good	Fairly firm	Very little	Good.
Gros Grandia	Poor	Very firm	Pronounced	Poor.
Haiti	Fair	Firm	Little	Fair.
Key West	Rather poor	Fairly firm	do	Poor.
Marneyita	Fair	Firm	do	Rather poor.
Miles	do	do	Pronounced	Poor.
Nancy Hall	Very good	do	Little	Very good.
Pierson	Fair	Very firm	do	Very poor.
Porto Rico	Very good	Fairly firm	do	Fairly good.
Pumpkin "Yam"	Fair	Firm	Pronounced	Fair.
Purple "Yam"	Poor	Very firm	do	Poor.
Red Bermuda	Fair to good	Firm	Little	Do.
Red Brazil	do	Fairly firm	do	Do.
Southern Queen	Rather poor	do	Pronounced	Rather poor.
Triumph	Good	Firm	Little	Poor.
Vineless Pumpkin "Yam"	Very good	do	do	Very good.
Vineless "Yam"	Fair	do	do	Poor.
White Sealy	do	do	do	Fair.
Yellow Belmont	Rather poor	Very firm	Pronounced	Poor.
Yellow Jersey	do	Fairly firm	Little	Do.
Yellow Strasburg	Fair to good	Firm	Pronounced	Fair.
No. 10412	Poor	do	do	Very poor.
No. 10650	Good	do	Little	Poor.
No. 11284	do	do	do	Fair.
No. 12686	Fair	do	do	Fair to good.
No. 24012	Fair to good	do	do	Poor.
No. 24013	Poor	do	do	Do.
No. 24014	Good	Very firm	do	Do.
No. 24015	Very poor	do	Pronounced	Do.

It is seen that of these 36 different varieties and strains of sweet potatoes approximately 70 per cent graded from fair to very good from the standpoint of attractiveness of the canned product; all were firm in consistency; about 70 per cent showed little tendency to discolor when exposed to the air after being removed from the can; and about 30 per cent of them graded from "fair" to "very good" with respect to table quality. Although attractiveness, tendency to remain bright on exposure to the air, and good table quality were not always found in those varieties showing one or more of these qualities, it is of interest to note that a considerable number of them did combine all of these qualities; as, for instance, Gold Skin, Nancy Hall, Porto Rico, and Vineless Pumpkin "Yam."

In this connection the results of the judging by the committee of canned-food specialists will be of interest. Although there was not complete agreement in all cases among individual judges as to the merits of the canned product of the different varieties, Nancy Hall was given first place by a large majority of the judges, with Gold Skin ranking second. With some, Vineless Pumpkin "Yam" was considered as second only to Nancy Hall, and favorable mention was made of Porto Rico, Big Stem Jersey, Triumph, Red Bermuda, and several others.

COOKING TESTS

To determine the suitability for culinary uses of the canned product from freshly dug sweet potatoes as compared with that from cured potatoes, a number of samples of selected varieties were submitted to the experimental kitchen of the Bureau of Chemistry for practical cooking tests, and the results were judged by a committee of food experts from that bureau. The samples submitted included both the whole and the pulped or pie-stock canned sweet potatoes of the freshly dug and the cured and stored Vineless Pumpkin "Yam," Gold Skin, and Triumph varieties, and the whole potato canned material of the Porto Rico. These were opened on the morning of January 5, 1923, and the following products and dishes prepared: (1) Pie fillers from the pie stock material of both the freshly dug and the cured and stored potatoes; (2) pies with crusts, from the freshly dug potato stock; (3) sliced and sautéd sweet potatoes from the freshly dug potato product (the cured potato product was too soft for slicing); (4) sliced and candied pie stock from the freshly dug potato product, except in the case of the Porto Rico, in which whole potato canned product was used.

For the preparation of pie fillers and pies it was found necessary to pass the canned material through a potato ricer because of the firmness of the product.

The results of these practical cooking tests are briefly described in the following summaries:

VINELESS PUMPKIN "YAM"

Pie filler.—Somewhat grained in texture but excellent in appearance and flavor. Filler prepared from the cured potato stock was smoother in texture.

Pies.—Rather coarse grained owing to the curdling of the milk during preparation and cooking. In all other respects the pies were satisfactory.

Sautéd potatoes.—Good, the slices remaining intact during the cooking and the product being very attractive and satisfactory.

Candied sweet potato.—The slices crumbled somewhat, but otherwise the product was excellent.

GOLD SKIN

Pie filler.—Very slightly grained but excellent in appearance and flavor. This filler would be very acceptable anywhere. Filler from cured potato stock was a little smoother in texture.

Pies.—Very good, somewhat smoother in texture than those from the Vineless Pumpkin "Yam."

Sautéd potatoes.—Very fine product, the slices remaining intact during the cooking. Appearance and flavor very satisfactory.

Candied sweet potato.—Excellent. Slices held their form perfectly and made a most pleasing and attractive dish.

TRIUMPH

Pie filler.—Good. Slightly grained in texture but not enough to be objectionable. The filler from the cured potato stock was a little smoother in texture.

Pies.—Good. Slightly grained in texture.

Sautéd potatoes.—Excellent. Slices held their form perfectly.

Candied sweet potato.—Very good. Slices held their form in the preparation and made an attractive dish.

PORTO RICO

Pie filler.—Good, but slightly grained in texture and showed some fibers which were somewhat objectionable. Filler from the cured potato stock was a little smoother in texture but the fibers were too much in evidence for the best results.

Pies.—Very satisfactory, though slightly grained.

Sautéd potatoes.—Satisfactory. Slices held their form perfectly although made from whole potato canned product.

Candied sweet potato.—A very attractive dish. Slices held their form perfectly.

In judging the merits of the different varieties in these cooking experiments the committee was nearly unanimous in giving first place to Gold Skin, with Vineless Pumpkin "Yam" and Porto Rico tying for second place.

From these studies it is seen that in the canned product from the freshly dug sweet potatoes the housewife has an excellent material for a number of attractive dishes, some of which can not be prepared satisfactorily from the sweet potatoes canned after curing and storage.

SUMMARY

The study herein reported has shown that during curing and storage profound transformations take place in the sweet potato, and further alterations occur during the cooking process.

The physical evidence of these transformations, as they are manifested in the canned product, are a very marked softening of the flesh of the potato and a very considerable increase in sweetness. In all of the varieties studied the freshly dug potatoes yielded a very firm product, whereas the cured and stored stock yielded a product varying all the way from a medium to a very soft consistency.

The chemical changes taking place are evidenced by a transformation of part of the starch to dextrin and sucrose, thus altering the starch moisture ratio, and during the cooking, a splitting of a portion of the starch to maltose. These changes are responsible for the altered physical consistency of the canned product and its increase in sweetness. The ratio of starch to moisture determines the consistency, and the chief factor causing the difference in sweetness is the amount of sucrose formed.

Practical cooking tests have shown that the canned product from freshly dug sweet potatoes is well adapted to a variety of table uses and, because of its firmness, is superior to that canned from cured stocks for the preparation of such dishes as sautéd and candied sweet potatoes.

Although many of the varieties studied gave an attractive product in the can, a few of them were found to have particular merit. Nancy Hall, Gold Skin, Vineless Pumpkin "Yam," and Porto Rico were among the best, but a very good canned product was obtained from Big Stem Jersey, Triumph, Red Bermuda, and others. It is important to note that those possessing the highest quality in the product of the freshly dug potatoes were also judged, in the earlier studies, as having particular merit when canned after the usual curing and storage treatment.

As a result of these combined studies it is apparent that by judicious handling of the raw stock the sweet-potato canner is able to put on the market products suited to every demand which the sweet potato supplies as a table commodity, and which for certain culinary uses is equal if not superior to the fresh vegetable.

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A LEAF SPOT OF CRUCIFEROUS PLANTS CAUSED BY ALTERNARIA HERCULEA [1]

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INTRODUCTION

While studying the ring spot of cruciferous plants caused by *Mycosphaerella brassicicola* (Fr.) Lindau, another type of leaf spot was found to be common, especially in a vegetable section on the western side of San Francisco Bay in California, just south of the city of San Francisco. The disease was especially prevalent on cabbage (Columbo variety) and cauliflower (50 varieties) growing in the eastern part of this section in the autumn and winter of 1923–24. Since two other similar leaf-spot diseases also occurred in this locality, some observations and studies were made for the purpose of distinguishing between them. The object of this paper is to record the results of these studies.

SYMPTOMS OF THE DISEASES

Ring spot is very common on crucifers in the vegetable-growing section above referred to, known as the Colma section. Although this disease is described elsewhere (*7*),[2] a brief description is given here for convenience.

Infection first becomes evident on the leaves, regardless of age, as very small dark-colored spots one-half millimeter or less in diameter. These spots enlarge, and in 3 to 4 weeks there are lesions 1 to 2 centimeters in diameter. Both the upper and lower surfaces of mature lesions are thickly studded with pycnidia or perithecia or both, they being more abundant on the upper surface. The pycnidia and perithecia are often arranged in concentric circles; hence the name ring spot. Plate 1, A, shows the characteristics of the lesions. These lesions vary in color, but are usually sepia to clay-colored. However the pycnidia and perithecia are often so abundant that the spots appear almost black. There is usually a rather sharp line of demarcation between the diseased and healthy tissues, although the tissues immediately surrounding the lesions may be yellow ochre, gradually shading off to the normal green color. Nearly all parts of the host are susceptible.

Another leaf spot found in this section on various cruciferous hosts is caused by the fungus commonly known in this country as *Alternaria brassicae* (Berk.) Sacc.[3] As stated in an earlier paper (*6*), this disease is not very prevalent in the San Francisco Bay section. However, it occurs at times and may be found on the same leaves with ring spot and the disease described below. This disease is known as

[1] Received for publication Feb. 8, 1926; issued October, 1926.

[2] Reference is made by number (italic) to "Literature cited," p. 650.

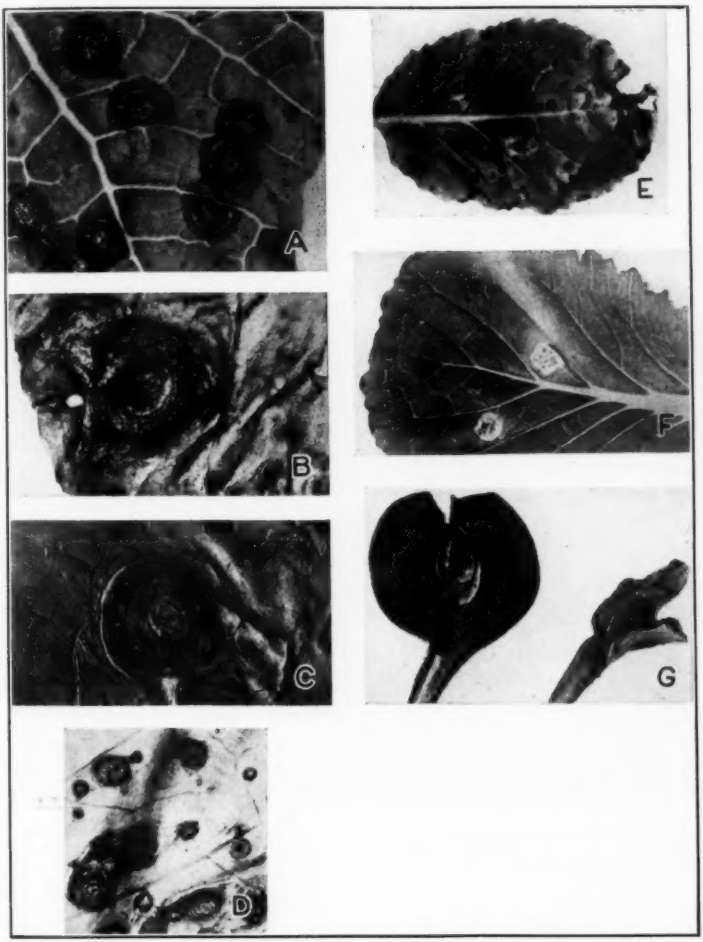
[3] The name of this fungus has recently been changed to *Alternaria circinans* (B. & C.) Bolle by Bolle (*2*). It is not the purpose of this paper to go into a detailed discussion of nomenclature, hence the question as to whether or not Bolle is justified in making this change will be left open.

black leaf spot (*4*), Alternaria spot, or black mold. In its early stages it is indistinguishable from the dark spot caused by the ring-spot fungus. The spots spread rather rapidly, forming circular lesions varying in size from 0.5 to 1 cm. in diameter. The color varies from grayish to brown. Under conditions of high humidity the surfaces of the lesions become covered with sporophores and spores of the causal organism, which give them an olivaceous appearance. As in the case of ring spot, these fruiting bodies are arranged in concentric rings (pl. 1, B).

The third type of leaf spot found in this locality resembles the other two in size, shape, distribution over the leaf surface, and in presence of concentric rings. These latter are clearly shown in Plate 1, C and D. For convenience in this discussion, this disease will be designated as gray leaf spot, to distinguish it from black leaf spot and ring spot. The disease differs from ring spot in that the lesions are usually lighter in color and bear no pycnidia or perithecia. It is similar to the black leaf spot, in that under moist conditions the spots are covered with an olivaceous growth of the causal fungus (pl. 1, C). However, this growth is usually not as dense as is the case on the black leaf-spot lesions, and under field conditions in this locality it usually is not seen at all. Likewise, the lesions of the gray leaf spot are, on the average, smaller and lighter in color than are those of the black leaf spot (pl. 1, D). Gray leaf-spot lesions on other hosts in California have much the same appearance as on cauliflower and cabbage, except for a slight variation in color. On Chinese cabbage, radish, and turnip, the spots are light gray, while on cabbage and cauliflower they vary from gray to dark brown. As seen in the field they are usually some shade of gray.

DISTRIBUTION AND ECONOMIC IMPORTANCE

This disease is not a new one to science, neither does it have a limited distribution. It is known to occur in central and southern California, and in Florida, New York, Alabama, New Jersey, Indiana, Ohio, and Connecticut, and it probably is present elsewhere. In spite of this prevalence it apparently has been given little attention by pathologists, no doubt because it ordinarily causes little damage. It was, however, very prevalent on cauliflower and cabbage in certain parts of the Colma section in the autumn of 1923 and the winter of 1924. The ring spot was abundant near the ocean, but became decreasingly prevalent back from the coast; and the reverse was true of the gray leaf spot. The lower leaves of cauliflower were quite badly spotted with the latter disease in some fields on the eastern side of the Colma section. No doubt some of the lower leaves were much debilitated by the disease and the heads correspondingly decreased in size. Nevertheless, it is very difficult to determine losses produced by diseases of this nature. For the most part only about six of the lower leaves of the large plants were attacked, and the plants produced fair-sized heads. In no case was the disease severe enough to justify control measures.



A.—Young cauliflower leaf affected with ring spot caused by *Mycosphaerella brassicicola*. The small black spots are lesions in early stages of development. 1.2×natural size.

B.—Portion of a cauliflower leaf showing a large lesion of black leaf spot caused by *Alternaria brassicae* 1.2×natural size.

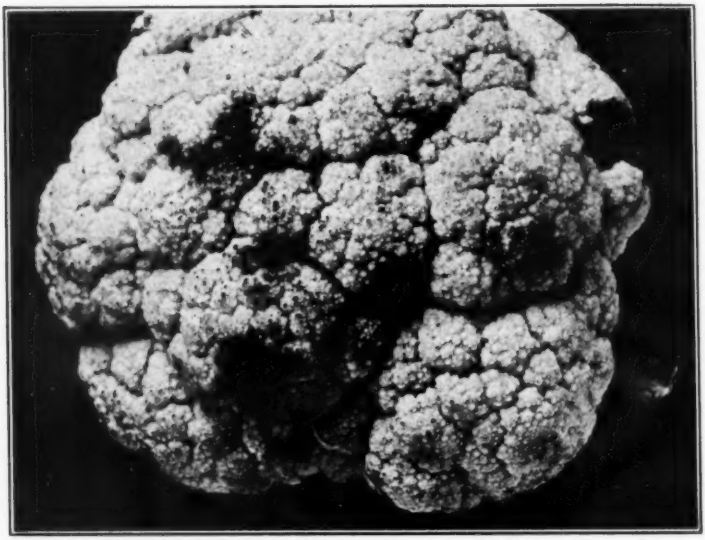
C.—Portion of cauliflower leaf showing a large lesion of gray leaf spot caused by *Alternaria herculea*. 1.2× natural size.

D.—Section of cauliflower leaf affected with gray leaf spot caused by *Alternaria herculea*. [This type of lesion is more common than that shown in C. The concentric rings in these lesions are more pronounced than in the case of ring spot (pl. 1, A]. The spots are usually smaller and lighter in color than are the black leaf-spot lesions (pl. 1, B). About half natural size

E.—Young cabbage leaf showing gray leaf-spot lesions as they appeared thirteen days after inoculation. About half natural size.

F.—Leaf of Chinese cabbage having two gray leaf-spot lesions produced by artificial inoculation. About half natural size.

G.—Cotyledons of cauliflower seedlings artificially infected by *Alternaria herculea*. 1.8×natural size.



A head of cauliflower photographed one week after it was inoculated with *Alternaria herculea* and placed at 20° to 25° C. in a moist chamber. The black spots show the extent of infected areas. Two-thirds natural size

THE CAUSAL ORGANISM

The gray leaf spot is caused by the fungus first described by Ellis and Martin (*3*) on horse-radish (*Armoracia rusticana* Gaertn.) as *Alternaria herculea* (Ell. & Mart.) Elliott. Later Stewart (*5*) reported it as being common on flat turnip and found occasionally on cabbage on Long Island, N. Y. The fungus was first classed as a Macrosportium, but was later transferred to the genus Alternaria by Elliott (*2*), where, according to the present conception of the genus, it undoubtedly belongs.[4]

Frequently it is necessary to make a microscopic examination of the spores before the black and gray leaf spots can be differentiated. Figure 1 shows how the spores differ in size and shape. The apical

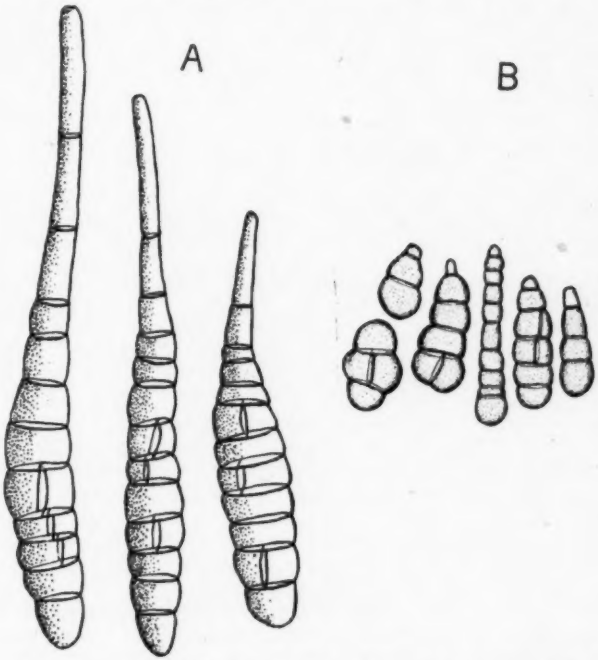


FIG. 1.—A. Three spores of *Alternaria herculea*. × 500. B. Group of spores of *Alternaria brassicae*. × 500

cell of an *Alternaria herculea* spore is characteristically long as compared with that of an *A. brassicae* spore. The spores of the former are also lighter in color, proportionately longer and narrower, and usually have more longitudinal septa than do those of the latter. *A. herculea* spores measure 16 to 28 × 125 to 225 μ, while the largest spores of *A. brassicae* (5 to 8 septate) are 9 to 16.8 μ wide by 35 to 75 μ long (*6*).

[4] Bolle (*1*) has decided that the correct name of this fungus is *Alternaria brassicae* (Berk.) Bolle. However, the present writer is not entirely convinced that this change is justified, so for the purpose of this paper he prefers to use the name *A. herculea*.

Stewart (5) states that this fungus is undoubtedly parasitic on turnips, but cites no experiments to prove it. Since no proof of its pathogenicity could be found, a few tests were made on different hosts. Isolations were made from a number of typical lesions on cauliflower and *Alternaria herculea* was obtained in nearly every case. Inoculation tests on cauliflower, cabbage, and Chinese cabbage showed this fungus to be parasitic on them. The fungus was isolated from Chinese cabbage and from rutabaga-infected cabbage. Not only were the old leaves of cabbage infected, regardless of size, but the cotyledons were also. Cauliflower cotyledons only a few days old have been collected a number of times from seed beds at Colma and the fungus isolated. *Alternaria herculea* isolated from rutabaga leaves was used to inoculate cauliflower seedlings growing in the greenhouse, in the following manner: Hyphae in small amounts were placed on the cotyledons without consciously injuring them, and the plants were covered with battery jars. Three days later there was evidence of infection in the form of small, dark spots just visible to the unaided eye. In 12 days some of the cotyledons were almost entirely killed, while others showed quite distinct grayish-brown lesions having the concentric ring character (pl. 1, G). These lesions were very similar to those found on the cotyledons under natural conditions. In like manner, hyphae, from a culture of *A. herculea* from rutabaga were placed on the moistened leaves of Chinese cabbage seedlings (Wong Bok) growing in the greenhouse. These plants were kept moist under a bell jar for three days. At the end of that time water-soaked spots varying in size with the amount of the inoculum were present on the leaves. Plate 1, F, is a photograph of two of these lesions as they appeared at the end of 12 days. These spots were circular in outline, grayish, and varied from 1 mm. to 1 cm. in diameter. There was usually a sharp line of demarcation between the diseased and healthy tissues. The fungus fruited sparingly on the lesions, except when they were placed under conditions of high humidity. The fungus isolated from rutabaga reinfected the leaves of seedlings of this host (improved purple top variety) as well as cauliflower seedlings held in the greenhouse and inoculated as in the instances cited above. The fungus was reisolated from the lesions formed on rutagaba and used to inoculate Chinese cabbage with positive results. There is, therefore, considerable evidence that the fungus attacking all of these hosts is the same.

In one experiment conducted in the Pathological Garden at Berkeley, Calif., and designed to prove the pathogenicity of the ring-spot fungus, cauliflower leaves heavily infected with ring spot and also having some gray leaf-spot lesions, were suspended over each of 12 half-grown cauliflower plants. There was a heavy fog at the time, and this, together with some rain at intervals, kept the plants wet most of the time for three days and nights. Likewise, similarly affected leaves were suspended over some cabbage seedlings growing near. Six days later numerous small dark lesions were present on many of the cauliflower as well as the cabbage leaves. These proved to be due to *Alternaria herculea*, the lesions caused by the ring-spot fungus not being distinguishable as such until more than a week later. Under the conditions of the test, the *A. herculea* lesions developed much more rapidly than did those of the ring-spot fungus. Some of the

A. herculea lesions on the cabbage seedlings are shown in Plate 1, E, as they appeared 13 days after inoculation.

Since *Alternaria herculea* is so commonly present in some of the cauliflower fields in the Colma district, and as this cauliflower is held for several days in refrigerator cars and on the market before it reaches the consumer, the question arises as to what effect this fungus may have on the curd. To determine this point the following experiment was conducted. A medium-sized head of cauliflower was washed under the tap, placed in a battery jar lined with moist filter paper, and inoculated by placing on it in several places small amounts of hyphae from an 8-day old culture of *A. herculea*. The battery jar was then covered with a glass lid and held at room temperature (20° to 25° C.). A very slight amount of decay was observed on the third day after the inoculations were made. The decay developed slowly, as may be inferred from the condition of the head at the end of a week, as shown in Plate 2. It appeared from this experiment that *A. herculea* can cause a decay of the curd; but, under the conditions of the experiment, the rot progressed very slowly. The character of this rot and the appearance of the lesions are very similar to those caused by *A. brassicae*, as described in a former paper (*6*). The decay, however, does not develop as rapidly as does that caused by the latter fungus.

Another experiment was then conducted to determine whether any decay would occur at temperatures more nearly approaching those existing in a refrigerator car. Four heads were inoculated as in the above experiment, and held at temperatures of 0°, 2°, 4.5°, and 9° C., respectively. Observations made a month later showed that at 0° the fungus had caused a decay extending 2 to 3 mm. in depth, and covering an area slightly larger than the inoculum. The decayed tissues were quite soft and moist and were lighter in color than the surrounding healthy tissues. At 2° the fungus had decayed about 1 c. c. of the tissues upon which the inoculum had been placed. The infected tissues were brownish, soft and moist, and a microscopic study showed them to be filled with mycelium penetrating in every direction. The cellular structure was almost entirely disorganized. A few isolated cells were seen, but for the most part the tissues had been replaced by the fungus. The decayed tissues had an odor common to decaying cauliflower, but not the foul odor characteristic of bacterial rots of this host. At 4.5° and 9° C. the amount of decay was only slightly greater than that at 2°. *A. herculea* was recovered in pure culture from the decayed tissues at all four temperatures. This experiment, although on a very limited scale, indicates that this fungus can decay the curd of cauliflower, even at temperatures similar to those to which the cauliflower is subjected in the refrigerator car. However, the fungus develops slowly and ordinarily will not cause much damage to cauliflower during the time it is in transit.

SUMMARY

A leaf spot of cruciferous plants caused by *Alternaria herculea* (Ell. & Mart.) Elliott, which was quite prevalent in the vegetable section just south of San Francisco, Calif., in the season of 1923–24 is described and illustrated. This disease is quite similar to the leaf spot

caused by *Alternaria brassicae*, and is no doubt frequently confused with it.

The parasitism of the fungus on cabbage, cauliflower, Chinese cabbage, and rutabaga was proved.

Alternaria herculea can cause a slow decay of the curd of cauliflower at temperatures of 0°, 2°, 4.5°, 9°, and 20° to 25° C.

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HERITABILITY OF DIFFERENT RATES OF SHEDDING IN COTTON[1]

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INTRODUCTION

The shedding of flower buds and young bolls of cotton has been the subject of several investigations in which shedding has been considered from the physiological point of view and with especial reference to the influence of environmental factors. A survey of the literature of this phase of the subject would be out of place here, but publications by Balls (*1, p. 67–75*[2]), Thornton (*23*), Lloyd (*15, 16*), Harland (*5*), Ewing (*4*), Cook (*3*), McKillop (*17*), Mason (*18, 19*), Jivanna Rao (*12*), Prescott (*22*), Zaitzev (*24*), and Hilson (*11*) may be cited in this connection.

Shedding has been little studied from the point of view of heredity, but there are published data which make it reasonably certain that genetic factors are involved. Harland (*5, p. 78*), summarizing observations made by him on the island of St. Vincent, W. I., states:

> In hybrids between types differing in the bud-shedding habit, it has been established that when sea island is crossed with a West Indian native in which the bud-shedding habit is developed, the F_1 exhibits this bud-shedding. * * * Crosses between upland and West Indian native give rise to an F_1 which does not shed its buds.

Ewing (*4, p. 64, 66*) found differences in the rate of shedding of different varieties of upland cotton. Cook (*3*) remarks:

> A genetic factor is plainly indicated in plants that abort all of their buds, while their neighbors mature good crops. Egyptian cotton may retain nearly all of its buds and young bolls while upland varieties in adjacent rows are shedding nearly all of their buds.

Patel (*20*), comparing six strains of a variety cultivated in India and referred by him to *Gossypium herbaceum* L., found that the three strains which gave the highest percentages of bud shedding in 1920–21 also gave the highest percentages in 1921–22. The one strain which was highest of all in bud shedding in both seasons also ranked highest in percentage boll shedding in three successive seasons.

The comparative boll shedding of the Pima variety, representing the Egyptian type of cotton, and of several varieties of the upland type, was studied by King, Loomis, and Varmette (*14, p. 943*) at the United States Field Station, Sacaton, Ariz. In this case the comparison is between different species, Egyptian cotton belonging to the group containing sea island cotton (*G. barbadense* L.) and the upland varieties belonging to *G. hirsutum* L. King and his coworkers state in regard to their findings:

> Pima cotton in 1922 retained 82.5 per cent of its bolls and in 1923 70.7 per cent, under conditions where upland varieties usually retained less than 30 per cent of their bolls.

[1] Received for publication Mar 19, 1926; issued October, 1926.
[2] Reference is made by number (italic) to "Literature cited," p. 669.

The existence of a difference of this magnitude between the two types is not surprising in view of the differences in tissue-fluid properties discovered by Harris and his coworkers (*6*, *7*, *8*, *9*) and the numerous and striking morphological differences between Egyptian and upland cottons, some of which have been summarized by Kearney (*13*, *p. 4–6*).

A study of hybrids between Pima Egyptian and Acala upland cotton, grown at Sacaton, Ariz., in 1925, has given additional evidence that there are genetic variations in shedding. The comparative shedding rates of the parental types will first be considered.

COMPARATIVE SHEDDING OF PIMA EGYPTIAN AND ACALA UPLAND COTTONS

Shedding records were kept on plants of Pima and of Acala cotton grown under as nearly as practicable identical conditions at the United States Field Station, Sacaton, Ariz., in 1923, 1924, and 1925, and at the United States Cotton Field Station at Shafter, Calif., in 1923.[3]

The records from which were computed the shedding percentages took account only of primary or extra-axillary buds and flowers borne on the fruiting branches of the main stalk. Secondary or axillary buds and flowers, such as appear at many nodes of the fruiting branches, were ignored, as well as all buds and flowers borne on the vegetative branches or limbs. The justification for this procedure is that individual plants differ greatly in the production of vegetative branches and of axillary flower buds on the fruiting branches, and the shedding of buds and young bolls borne in these positions seems to be more readily affected by slight variations of the environment than is the case with those borne in the extra-axillary position on the fruiting branches of the main stalk.[4]

The seasonal shedding percentages, as given in Table 1, were computed from the total number of flower buds produced and shed and the total number of flowers produced and shed (as young bolls) by all plants in each population, taken as one array. The standard deviations and probable errors of the mean percentages were computed from the squared departures of the individual plant percentages from the mean percentage of the whole population, each squared departure having been weighted for number of buds or of flowers on the plant in question.

It is evident from the data in Table 1 that the percentage of bud shedding (abscission before anthesis) of the two types did not differ significantly either in 1924 or in 1925. But in percentage of boll shedding (abscission after anthesis) the two types differed greatly and very significantly in all four comparisons. The Acala percentage was from 4.2 to 7.4 times the Pima percentage and the difference was from 14 to 35 times its probable error.

[3] The records were kept at Sacaton in 1923 by Max Willett and Dow D. Porter, of the Office of Alkali and Drought Resistant Plants; at Shafter in 1923 by Robert H. Peebles, of the same office; and at Sacaton in 1924 and 1925 by Harold F. Loomis, of the Office of Acclimatization and Adaptation of Crop Plants. The writers are indebted to O. F. Cook for the use of Mr. Loomis's data.

[4] C. J. King has called the writers' attention to the fact that in the Pima variety, at least, the effective shedding, as shown by the number of vacant nodes on the fruiting branches at the end of the season, is much less than is indicated by the shedding percentages based on extra-axillary buds only, since bolls develop from secondary or axillary buds at many of the nodes from which the primary or extra-axillary bud had been recorded as shed. This observation is important from the point of view of ultimate yield but does not affect the validity of the comparison of the two types of cotton in this paper.

TABLE 1.—*Seasonal shedding percentages of Egyptian cotton (Pima variety) and upland cotton (Acala variety) in 1923, 1924, and 1925* [a]

Variety	Year	Station	Bud shedding			Boll shedding		
			Number of plants	Number of buds	Percentage shed	Number of plants	Number of flowers	Percentage shed
Pima	1923	Sacaton, Ariz.	20	1,317	39.4±0.89	40	1,565	15.5±1.48
Acala	1923	do				40	2,340	69.4±1.37
Difference								53.9±2.02
Pima	1923	Shafter, Calif.	20	955	52.1±1.03	30	590	9.3±1.35
Acala	1923	do				40	1,240	68.9±1.01
Difference								59.6±1.69
Pima	1924	Sacaton, Ariz.	20	1,445	37.4±0.99	20	904	19.1±1.71
Acala	1924	do	20	1,884	36.2±1.36	20	1,202	80.7±1.11
Difference					1.2±1.68			61.6±2.04
Pima	1925	Sacaton, Ariz.	20	1,791	43.9±1.19	20	1,004	11.2±3.62
Acala	1925	do	20	1,796	47.4±1.27	20	945	66.7±1.83
Difference					3.5±1.74			55.5±4.06

[a] Bud shedding was not recorded on the Acala plants in 1923.

COMPARATIVE SHEDDING IN F_1 AND F_2 OF THE HYBRID PIMA × ACALA

The material representing this interspecific hybrid consisted of 10 first-generation and 91 second-generation plants, all grown in the same plot at the United States Field Station, Sacaton, Ariz., in 1925. The F_1 plants were near the south end of row 1. Of the F_2 plants 68 were scattered through the entire length of row 2, and 23 were in the south one-third of row 3. As was the case in recording the shedding of the parental types, only the primary or extra-axillary flower buds borne on the fruiting branches of the main stalk were considered.

All flowers opening during the period from June 17 to September 8 (84 days) were tagged daily and a daily record of bolls shed was kept for each plant. From these data the seasonal boll-shedding percentage (bolls shed as a percentage of total flowers) was computed for each plant. After the daily recording was terminated, counts were made on each plant of the total number of nodes on the fruiting branches of the main stalk and of the number of nodes at which the primary or extra-axillary bud or boll was missing. Subtraction from the latter number of the number of nodes at which shedding had occurred after anthesis, as shown by the daily records of boll shedding, gave the number of buds shed before anthesis, and this number, relative to the total number of nodes on the fruiting branches, gave the seasonal percentage bud shedding for each plant.

The frequency distributions of the shedding percentages (in classes of 5 per cent each) of the F_1 and F_2 populations are given in Table 2 and are shown graphically in Figure 1. The mean shedding percentages and their standard deviations, for each population, are stated in Table 3. The means were computed from the percentages of the individual plants, not from the distributions by classes as

given in Table 2. The standard deviations and probable errors were computed from the departures of the individual plant percentages from the mean percentage of the population.[5]

TABLE 2.—*Frequency distributions of percentage of bud shedding and percentage of boll shedding on 10 F_1 plants and 91 F_2 plants of Pima×Acala*

Percentage	Number of plants				Percentage	Number of plants			
	Bud shedding		Boll shedding			Bud shedding		Boll shedding	
	F_1	F_2	F_1	F_2		F_1	F_2	F_1	F_2
0–5					51–55		5		11
6–10	4	2			56–60		1		8
11–15	5	8		2	61–65				6
16–20		7	2	5	66–70		1		5
21–25	1	8	4	3	71–75				2
26–30		14	3	6	76–80				4
31–35		12	1	10	81–85		1		
36–40		11		7	86–90				2
41–45		10		10	91–95				
46–50		11		9	96–100				1

TABLE 3.—*Mean bud and boll shedding percentages and their standard deviations in F_1 and F_2 populations of Pima×Acala*

Number of plants	Generation	Bud-shedding percentage		Boll-shedding percentage	
		Mean	Standard deviation	Mean	Standard deviation
10	F_1	12.9±1.42	6.66±1.00		5.64±0.85
91	F_2	27.2±1.28	18.17± .91	23.7±1.20	18.30± .91
				47.2±1.29	
Difference		14.3±1.91	11.51±1.35	23.5±1.76	12.66±1.24
D/E [a]		7.5	8.5	13.3	10.2

[a] Difference divided by its probable error.

A comparison of the mean shedding percentages of the hybrids (Table 3) with those of the parent varieties in 1925 (bottom section of Table 1) is not very satisfactory, owing to the fact that the parental and the hybrid populations were grown in different fields. It is true that the plants of all four populations made an excellent growth, but the fact that the bud shedding of both Pima and Acala was approximately 45 per cent, whereas in F_1 and F_2 of the hybrid it was only 13 and 27 per cent, respectively, indicates a radical difference in the conditions under which the parents and hybrids were grown. The relative boll shedding of the four populations is more in line with the expectation, the mean percentages having been: Pima, 11; F_1, 24; F_2, 47; Acala, 67. In this case the means of both hybrid generations were between those of the parents.

[5] The standard deviations of the mean percentages have been increased by using Pearson's correction for the standard deviation of small numbers (*21, p. 529*). A further increase of the standard deviation has been made on the basis suggested by Collins and Kempton (*2*), who present the matter as follows: "Since the fluctuations in ratios due to errors of sampling become smaller as the ratio departs from 0.5 and the change is measured by the change in $\sqrt{pq}$, the observed standard deviations have been multiplied by $\frac{.5}{\sqrt{pq}}$. This changes the standard deviation to that expected had the ratio been 0.5 or 50 per cent." The probable errors were computed from the standard deviations as thus corrected.

Comparing the mean shedding percentages of the two hybrid generations as given in Table 3 it is seen that the rate of shedding, both of buds and bolls, was approximately twice as great in F_2 as in F_1. The difference F_2-F_1 is very significant in both cases, having been, respectively, 7.5 and 13.3 times its probable error. Comparison of the standard deviations of the two generations shows F_2 to have been nearly three times as variable as F_1 in bud shedding and more than three times as variable in boll shedding. Here again the difference F_2-F_1 is very significant, having been, respectively, 8.5 and 10.2 times its probable error.

The F_2 frequency distributions for percentage bud shedding and percentage boll shedding (Table 2 and Figure 1) give no indication of segregation in definite ratios, the presumption being that several genetic factors are involved. Shedding therefore behaves like most of the morphological characters in Egyptian–upland cotton hybrids (*13, pp. 15–26, figs. 3–41*), and the inheritance is of the type usually observed in size characters.

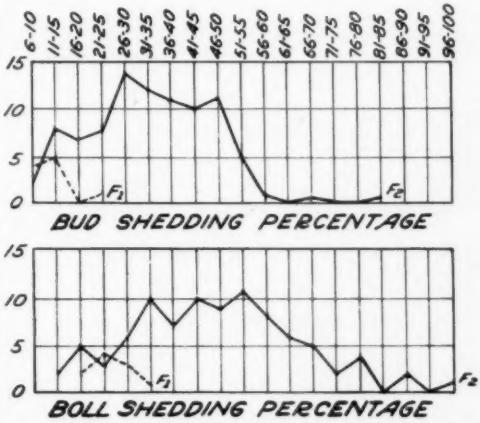

FIG. 1.—Frequency distributions of percentage of bud shedding and percentage of boll shedding of 10 F_1 plants and 91 F_2 plants of the cotton hybrid Pima×Acala. The figures on the ordinate indicate the numbers of plants in the respective class, and the figures on the abscissa indicate the shedding percentages in classes of 5 per cent each. The dotted lines represent the F_1 distributions and the solid lines the F_2 distributions

A strong presumption that there was Mendelian segregation in respect to shedding is created by the fact that the second generation was so much more variable than the first. The individual bud-shedding percentages ranged from 6 to 82 in F_2 but only from 8 to 22 in F_1. The boll-shedding percentages ranged from 13 to 100 in F_2 but only from 16 to 31 in F_1. In respect to both percentages the standard deviation was approximately three times as great in F_2 as in F_1.

Further evidence of segregation might have been had by comparing the shedding performance of the two hybrid generations with that of the inbred Pima and Acala families to which the parents of the hybrid belonged. Small progenies of both families were grown in 1925, but through an oversight shedding data were not obtained from them. Comparison with the Pima and Acala populations which afforded the data given in the bottom section of Table 1 would be of no use in this connection, because these populations were of the general commercial stocks of these varieties and, moreover, were grown in a different field from that in which the hybrid populations were located.

It has been mentioned that whereas the F_1 plants were located near the south end of row 1, the F_2 plants were distributed throughout the

length of row 2 and the south one-third of row 3. Because of the recognized importance of soil heterogeneity at the Sacaton station (*10, p. 609–612*), this wider distribution of the plants may well have been a factor in the much greater variation in the F_2. To test this point, the statistical constants were separately computed for 12 F_2 plants which were located at the south end of row 2 and occupied a section exactly coterminous with that occupied by the 10 F_1 plants in the adjacent row 1. The constants for this portion of the F_2 population, in comparison with F_1, are given in Table 4.

TABLE 4.—*Comparison of the statistical constants of the F_1 population and of a portion of the F_2 population of Pima × Acala, the plants of the two generations occupying adjacent and coterminous sections of rows*

Shedding percentages	Generation	Mean	Standard deviation
Percentage of bud shedding	F_1	12.9±1.42	6.66±1.00
	F_2	15.7±2.29	11.75±1.61
	Difference	2.8±2.69	5.09±1.89
	D/E [a]	1.0	2.7
Percentage of boll shedding	F_1	23.7±1.20	5.64± .85
	F_2	48.8±2.08	10.71±1.47
	Difference	25.1±2.40	5.07±1.70
	D/E	10.4	3.0

[a] Difference divided by its probable error.

A comparison of the means of these 12 F_2 plants with those of the whole F_2 population of 91 plants (Table 3) shows that the mean percentage of bud shedding is little more than half as great as in the whole population and the difference is significant (D/E 4.4). On the other hand, the mean percentage boll shedding of the sample does not differ significantly from that of the whole F_2 population.

The standard deviation of the mean bud-shedding percentage is only 65 per cent and that of the mean boll-shedding percentage is only 59 per cent as great in the sample as in the whole F_2 population. This fact indicates that soil heterogeneity may have been responsible for part of the variability in F_2 shown by the frequency distributions in Table 2 and the standard deviations in Table 3.

Returning to the comparison of adjacent plants of the two generations, as set forth in Table 4, in which comparison soil heterogeneity presumably is eliminated, the mean bud-shedding percentage is not significantly higher in F_2 than in F_1, but the mean boll-shedding percentage is very significantly higher in F_2, the difference being 10.4 times its probable error. The standard deviations of the mean percentages show F_2 to have been about twice as variable as F_1, both in bud shedding and in boll shedding, although the differences are barely significant. But in view of the small numbers of the populations and the resulting large probable errors of the standard deviations, significant differences were hardly to have been expected.

While the position of the F_1 plants in an outside row may have given them a slight advantage, the adjacent F_2 plants made an excellent growth and it is unlikely that this difference in environment could have been a material factor in the heavier rate of boll shedding and in the greater variability in both bud shedding and boll shedding of

the F_2 population. On the whole, the evidence from this closer comparison of F_1 and F_2 does not seriously weaken the conclusion that genetic factors are involved in the shedding performance of this hybrid.

GENETIC VARIATION SHOWN BY COMPARING ADJACENT F_2 PLANTS

Further evidence as to whether genetic factors are concerned in shedding was sought by comparing the shedding percentages of pairs of F_2 plants, the two plants in each comparison having been adjacent in the row. This procedure virtually eliminates the influence of soil heterogeneity. The Pima × Acala F_2 population afforded 56 such comparisons, involving 79 of the 91 plants.[6] Significant differences (difference three or more times its probable error) were shown by 30 of the 56 pairs in percentage of bud shedding and by 28 pairs in percentage of boll shedding.

A means of expressing concisely what may be termed the nonfortuitous variation in a population is afforded by comparing the standard deviation computed from the mean squared differences between pairs with the standard deviation expected if only chance variation had occurred. Since in the observed standard deviation as thus computed the effect of soil heterogeneity is virtually eliminated, the amount by which it exceeds the expected standard deviation may be taken to represent the amount of variation due to genetic factors.

The observed and expected standard deviations were computed for the percentages of bud shedding and boll shedding in the Pima × Acala F_2 population of 1925. Also, for comparison, these standard deviations were computed for a pouplation of 100 plants of Pima cotton, consecutive in the row, on which shedding was recorded at Sacaton in 1922. Eleven of the 99 pairs of Pima plants differed significantly in respect to bud shedding and 10 pairs in respect to boll shedding.

The standard deviations based upon these comparisons of paired plants are given in Table 5.

In Pima × Acala F_2 the observed standard deviation is about 3 times the expected standard deviation, for both the bud-shedding and the boll-shedding percentage, and the difference in both cases is highly significant, being about 10 times its probable error. It is clear that the individual differences in shedding in this population are largely of a genetic nature.

In the relatively homogenous Pima population, on the other hand, the observed standard deviation only slightly exceeds the expected standard deviation for the bud-shedding and boll-shedding percentages, but the difference in each case appears to be significant (about 3.5 times its probable error).[7]

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[6] Numerous plants on which flowers had been bagged for self-fertilization were scattered through the row and these were not used; hence the reduced number of comparisons.

[7] The correlation of adjacent plants in respect to percentage of bud shedding was positive in both populations. In Pima×Acala F_2 the coefficient was not significant, having been $+0.167 \pm .087$ (1.9 times the probable error). It is not unlikely that the pronounced differences of a genetic nature in this case obscured the effect of soil heterogeneity. In the Pima population the coefficient was $+0.201 \pm .065$ (3.1 times the probable error). The low correlations in both populations indicate that soil heterogeneity was not very pronounced where they were grown.

TABLE 5.—*Observed and expected standard deviations of the mean shedding percentages in Pima × Acala F_2 of 1925 and in a Pima population of 1922*

Standard deviation	Pima × Acala F_2 (79 plants, 56 paired comparisons)		Pima (100 plants, 99 paired comparisons)	
	Mean percentage of bud shedding	Mean percentage of boll shedding	Mean percentage of bud shedding	Mean percentage of boll shedding
Observed [a]	15.11±0.96	20.38±1.30	5.32±0.255	5.32±0.255
Expected [b]	4.93	7.10	4.40	4.46
Difference	10.18±.96	13.28±1.30	.92±.255	.86±.255
D/E	10.6	10.2	3.6	3.4

[a] The formula is: $\sigma=\sqrt{\frac{\Sigma\left(d^2\times\frac{1}{E^2}\right)}{\Sigma\frac{1}{E^2}}}\div\sqrt{2}$ Where d=the difference between members of a pair and E=the probable error of the difference.

[b] The formula is: $\sigma=\sqrt{\frac{pq}{H}}$, where p is the observed shedding percentage of the population (as one array) q=100−p and H the harmonic mean number of buds (or flowers). H is computed by the formula $\frac{1}{\Sigma\frac{1}{n}/N}$ where n is the number of squares (or flowers) on each plant and N the number of plants involved in the series of pairs

CORRELATION BETWEEN BUD SHEDDING AND BOLL SHEDDING

Correlating, for the 91 plants of Pima × Acala F_2, grown in 1925, the percentage bud shedding with the percentage boll shedding, a value for r of $+0.201\pm.068$ was obtained. The coefficient is doubtfully significant, being slightly less than three times its probable error. The bud shedding and boll shedding percentages of 100 Pima plants at Sacaton, Ariz., in 1922 and of 44 Pima plants at Shafter, Calif., in 1922 gave coefficients of correlation of $-0.116\pm.067$ and $-0.231\pm.096$, respectively, neither coefficient being significant.

A negative correlation between bud shedding and boll shedding might be expected on physiological grounds, since if a certain amount of self-pruning is assumed to be necessary, heavy shedding of buds ought to favor the retention of most of the young bolls. On the other hand, a positive correlation might be expected on genetic grounds, since plants possessing factors conducive to abscission would probably show this tendency both before and after anthesis. Although none of the above coefficients of correlation appears to be significant with respect to its probable error, the fact that the two Pima populations of 1922 gave negative correlations indicates that here the physiological effect outweighed the genetic, while the positive correlation shown in F_2 of the Pima × Acala hybrid denotes a greater influence of genetic than of physiological factors in this population.

SUMMARY AND CONCLUSIONS

An interspecific cotton hybrid, Pima Egyptian × Acala upland, was studied for the purpose of ascertaining whether genetic factors are involved in the shedding of the flower buds and the young bolls (abscission before and after anthesis).

Comparison of populations representing the parental types showed practically no difference in the mean percentage of bud shedding but a consistently much higher rate of boll shedding in Acala than in Pima.

In both the first and second generations the hybrid gave a much lower mean percentage of bud shedding than either parental population, while the mean percentage of boll shedding of the hybrid in both generations was between the mean percentages of the parental types.

Comparing the two generations of the hybrid, the mean shedding percentage, both of buds and of bolls, was about twice as great in F_2 as in F_1 and the differences were very significant. Comparison of the standard deviations of the mean percentages showed F_2 to have been approximately three times as variable as F_1 and these differences also were highly significant.

Part of the greater variability in the second generation of the hybrid may have been due to the fact that the much larger F_2 population was exposed to a greater range of soil variation than the F_1 population. When this factor was eliminated by comparing the F_1 population with an approximately equal number of F_2 plants grown under the same soil conditions, the difference between the variability of the two generations was reduced but the standard deviations of F_2 were still about twice as great as those of F_1.

The frequency distributions of the F_2 population for percentage of bud shedding and percentage of boll shedding, give no indication of segregation in definite ratios. The behavior, which resembles that of most size characters in plants and animals, may be interpreted on the theory that three or more genetic factors are involved. That Mendelian segregation nevertheless occurred, is suggested by the fact that the variation was much greater in F_2 than in F_1.

Further evidence of genetic factors in shedding was obtained by comparing the shedding percentages of pairs of adjacent F_2 plants, the influence of soil heterogeneity being practically eliminated by this procedure. With 56 such pairs, significant differences were found in 30 cases in respect to bud shedding and in 28 cases in respect to boll shedding.

A comparison of the observed standard deviation computed from the mean squared differences between pairs with the standard deviation expected if only chance variation had occurred, showed that in Pima $\times$ Acala F_2 the observed was about three times the expected standard deviation in both bud shedding and boll shedding and the differences in both cases were highly significant; whereas, in a population of 100 plants of the Pima variety the observed standard deviation was only 1.2 times the expected standard deviation both in bud shedding and in boll shedding. The great disparity between the size of the observed and the expected standard deviations of the hybrid is convincing evidence of genetic differences among the individual F_2 plants.

The correlation between the percentage of bud shedding and the percentage of boll shedding of the Pima $\times$ Acala F_2 population was found to be positive, although scarcely significant, while in two Pima populations very small and probably not significant negative correlations were found. A positive correlation would be expected on genetic grounds, since genetic factors conducive to a heavy rate

of shedding presumably would operate both before and after anthesis. On the other hand, from a physiological point of view a negative correlation would be anticipated, since, assuming a certain amount of self-pruning to be necessary, a heavy rate of shedding before anthesis should be favorable to the retention of a greater proportion of the young bolls.

Differences in the rate of shedding of different types and varieties of cotton have been pointed out by previous investigators. Such differences indicate that shedding is, in part, a genetic phenomenon. The investigation described in this paper supplied the evidence that there are genetic factors for shedding which segregate and recombine in the usual manner.

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INFECTION AND TEMPERATURE RELATIONS OF BLACK ROT OF SWEET POTATOES IN STORAGE [1]

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INTRODUCTION

Black rot of sweet potatoes (*Ipomoea batatas*) is of considerable economic importance as a storage disease and may be responsible for some loss in transit and in the market when conditions are favorable for infection and development.

Black rot (*Ceratostomella fimbriatum* (E. & H.) Elliot) has its origin in the field, but the spores of the fungus may be carried into the storage house on the roots of the potato. This disease can be largely eliminated from the field through seed selection, disinfection, rotation of crops, and changing of the soil and manure (when manure is used for the purpose of heating) in the seed bed. Since, however, the disease is probably present in every region where sweet potatoes are grown, it continues to be a source of loss in the storage house and in the markets.

Where loss occurs in storage it may range from a fraction of 1 per cent to nearly 100 per cent. Poole [2] estimated a storage loss of 75 per cent in some instances where the field losses were confined to a few scattered hills. No figures to show the total loss throughout the country are at present available. In fact there seems to be no practical method known by which this loss can be accurately determined.

Although sweet potatoes may show little or no sign of black rot at digging time, the disease may develop and spread in the storage house.[3] The rapidity with which this occurs depends upon the agencies by which it is spread and the conditions in the storage house and the railroad car. The means by which it is spread have not been studied, but the following are listed as probable: Contact, air movement, rodents, insects, and man. Temperature and humidity are among the most important factors affecting its development.

In the present paper a consideration is given to the method of infection, the influence of temperature on infection, the development of the disease, and the growth of the pathogene in culture.

MATERIALS

A pure-line strain of *Ceratostomella fimbriatum* was employed in these experiments. It was grown on corn meal and incubated at room temperature.

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[1] Received for publication Apr. 27, 1926; issued October, 1926.

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Little Stem Jersey was the variety of sweet potato used. The potatoes were cured at temperatures ranging from 25° to 30° C. and stored at temperatures between 10° and 15°.

EXPERIMENTAL DATA

The data will be presented under seven headings: (1) Growth of the pathogene, (2) method of infection, (3) time required for infection, (4) influence of temperature on the development of the disease, (5) influence of time on the development of the disease, (6) influence of temperature and time on the number of infection, and (7) temperature limits of infection.

GROWTH OF THE PATHOGENE

Erlenmeyer flasks (200 c. c. capacity) of sweet-potato agar were inoculated with a water spore suspension. By means of a platinum loop a small drop of the suspension was placed at the center of the culture medium in each flask. The flasks were then placed in infection chambers [4] at different temperatures. The cultures were allowed to grow until those at the optimum temperatures had nearly reached the margin of the medium, so as to permit as much growth as possible at the border temperatures. This arrangement was desirable in order to compare the growth of the organism over a wide range of temperatures at a given time. It was not possible to bridge the entire temperature growth range with the size of flasks used. At 12° C. and below and at 34.5° and above little or no growth occurred in 16 days, the time required for the organism almost to cover the culture medium at the optimum temperatures. The cultures held at these temperatures were retained until a later date to allow for any further development.

The measurements were made by means of special calipers devised for that purpose.[5] They consisted of two metal plates drawn out to a point at the ends and fastened at the centers, so that they could open and close like a pair of scissors. These were inserted in the flasks when closed and then spread out so that the two points were at opposite margins of a colony. The distance was then measured between the points at the opposite end of the calipers.

The results recorded in Table 1 represent the average of two experiments. No growth took place at 40° C. in 16 days and none at 36° in either 16 or 40 days. Some growth occurred at 34.5° in 16 days. This temperature is very near the maximum, as is indicated by the large decrease in growth which occurred as the temperature was raised from 33.5° to 34.5°. There was growth in 5 of the 12 cultures at 33.5° in 16 days. Two of the cultures showed considerable growth and three but little. These three, together with the seven which had shown no growth, were retained at the same temperature for 24 days longer. At the end of the 24 days two of the three were contaminated, but the third showed an area of only 2 square mm. Two of the seven were also contaminated, but the other five showed no growth.

[4] LAURITZEN, J. I., and HARTER, L. L. SPECIES OF RHIZOPUS RESPONSIBLE FOR THE DECAY OF SWEET POTATOES IN THE STORAGE HOUSE AND AT DIFFERENT TEMPERATURES IN INFECTION CHAMBERS. Jour. Agr. Research 24: 441-456, illus. 1923.

[5] The calipers were devised by George F. Taylor, of the Office of Horticulture, U. S. Department of Agriculture.

TABLE 1.—*Growth of Ceratostomella fimbriatum on sweet-potato agar at different temperatures*

Number of flasks inoculated	Number of colonies measured	Length of growth period	Temperature	Average area of lesions	Remarks
		Days	*° C.*	*Sq. mm.*	
5	0	16	40.0	0	No growth in 3 flasks; 2 contaminated.
12	0	16	36.0	0	
5	1	16	34.5	5	
12	5	16	33.5	155	No growth in 7 flasks; growth in 5.
12	12	16	31.5	950	
17	16	16	28.5	1,782	
10	8	16	27.0	1,951	2 cultures contaminated.
16	15	16	24.5	1,929	1 culture contaminated.
16	14	16	23.0	1,884	2 cultures contaminated.
16	12	16	20.0	1,212	4 cultures contaminated.
16	12	16	18.5	766	Do.
10	9	16	14.0	61	
11	----------	16	12.0	----------	1 to 2 mm. of growth in all cultures.
11	----------	16	9.5	0	
11	----------	16	8.0	0	
11	----------	40	36.0	0	No growth in 9 cultures; 4 cultures contaminated.
10	1	40	33.5	2	No growth in 5 cultures; 4 cultures contaminated.
11	8	55	12.0	65	3 cultures contaminated.
11	0	55	9.5	0	No growth in 9 cultures; 1 contaminated; 1 doubtful colony.
11	0	55	8.0	0	
5	1	73	14.0	3,019	4 cultures contaminated.
5	2	73	11.5	647	2 cultures contaminated.
5	0	73	10.0	----------	Just a speck in 2; 1 culture contaminated; no growth in 2.

The lowest temperature at which there was any evidence of growth was 9.5° C. The growth at 10° was scarcely visible after 73 days. The greatest growth in 16 days occurred at 27° C., but it was only slightly greater than that at 24.5°. Some variation was noted in the results of the two experiments, the greatest growth in one occurring at 28° and in the other at 27°. The difference in the amount of growth which occurred between 23° and 28.5° was small. It may be said, therefore, that the optimum is represented by this range of temperature.

There was a marked increase in the growth of the fungus with the rise in temperature from 14° to 23° C. This augmented growth was due to two factors, namely, temperature and the area of the medium exposed to the action of the fungus. Since the growth of a colony takes place chiefly at the periphery, the area of growth is closely proportional to the circumference as long as the margin of the medium is not reached. Since the colony is larger at the higher temperature at any moment after growth begins, the area of growth is larger.[6] This difference in area is progressively augmented with the lapse of time. Thus we have two variable factors, varying in the same direction and combining in a greatly increased rate of growth with a rise in temperature. This relation operates in the opposite direction as we pass from the optimum temperature to the maximum. The range of temperature, however, is narrower. The relation of growth of the fungus to rise in temperature is graphically represented in Figure 1.

METHOD OF INFECTION

The data relating to the method of infection were obtained from two experiments. In the first the potatoes were inoculated by

[6] LAURITZEN, J. I., and HARTER, L. L. THE INFLUENCE OF TEMPERATURE ON THE INFECTION AND DECAY OF SWEET POTATOES BY DIFFERENT SPECIES OF RHIZOPUS. Jour. Agr. Research 30: 793-810, illus. 1925.

dipping in a spore suspension of the fungus. They were then placed in battery jars and distributed to various temperatures. After 17 days 123 lesions on potatoes that had been exposed to various temperatures were examined to determine whether or not infection had taken place through the uninjured skin. In all doubtful cases they were examined under the hand lens to make sure of the presence or absence of defects at the point of infection. Seven lesions showed a complete absence of any sign of defect or disturbance in the skin at the point of infection; 2 showed very slight abrasions; 70 showed

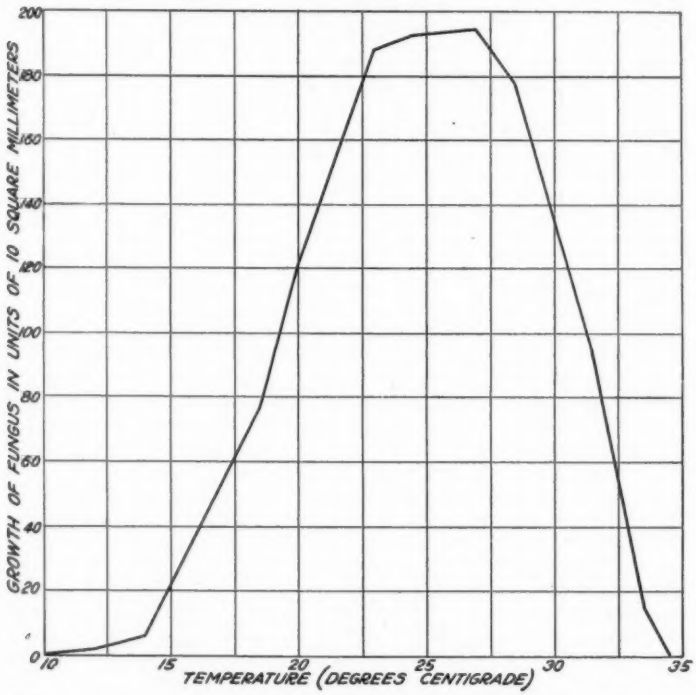

FIG. 1.—Curve showing the increments of growth of *Ceratostomella fimbriatum* with the rise in temperature after 16 days

abrasions or apparent disturbances at the center. In 41 cases infection occurred where rootlets emerged from the potatoes and in 3 cases it occurred through wounds.

In the second experiment the potatoes were inoculated as in the first, but were stored at various temperatures in wire baskets instead of in battery jars. One hundred and thirty-six lesions were examined. Infection appeared to have occurred through the uninjured skin in 6 cases. There were definite abrasions or disturbances in the surface tissue of 119 lesions. Infection occurred through rootlets in the remaining 11 cases.

The results of these experiments indicate that infection may sometimes take place through the uninjured skin. It would seem that

as a rule the pathogene penetrates the tissue of the host where the skin offers the least resistance, although, of course, it is possible that the slight abrasions or defects in the skin may have been developed as a result of the action of the fungus.

TIME REQUIRED FOR INFECTION

Infection had occurred at temperatures of 23°, 24°, and 27°C. in 4 days; at 31°, 28°, and 20° in 5 days; at 18.5° in 6 days; and at 14° in 8 days. Judging by the size of the lesions, infection occurred at the various temperatures in the following order: 25° (3.4 mm. diameter), 28° (2.6 mm.), 23° (2.1 mm.), 31° (1.9 mm.), 20° (1.4 mm.). These measurements are the average diameters of 5 spots in each case at the end of 5 days. The average diameter of 5 lesions after 8 days at 14° was 1.4 mm. and at 18° 3.3 mm. The time required for infection to become evident varied from 4 to 8 days over a range of temperatures from 14° to 31°.

INFLUENCE OF TEMPERATURE ON THE DEVELOPMENT OF THE DISEASE

Various methods were employed to measure the influence of temperature on the development of disease. In the first experiments the potatoes were inoculated by dipping in a spore suspension. They were then maintained at various temperatures for a period of 21 days. After 13 and 21 days the diameters of the lesions were measured by means of a caliper. Two methods of estimating the quantity of decay present at the various temperatures were employed. First, the diameter of the lesions was measured, and second, the area of the lesions was determined. The data were computed from two experiments by selecting the 10 largest lesions at each temperature in each experiment and striking an average of the 20 lesions. The area was determined by computing the area of each lesion and striking an average of the 20 areas. The use of the largest lesions that develop at each temperature as a standard of measurement is open to criticism, because in some instances (at the border temperatures) the number of lesions may have been too small for comparison with those where there was a larger number to select from. Moreover, by this method the amount of decay that occurs at all temperatures is exaggerated. However, the general relations found are fairly comparable to those obtained by other methods.

TABLE 2.—*Influence of temperature on infection of sweet potato by black rot and development of the disease after 13 and 21 days*

Temperature	Diameter of lesions after—		Area of lesions after—	
	13 days	21 days	13 days	21 days
°C.	*Mm.*	*Mm.*	*Sq. mm.*	*Sq. mm.*
34.4	0	0	0	0
32.0	4.4	7.6	15	48
29.0	10.0	17.1	82	232
24.3	11.2	17.8	98	254
23.0	11.2	16.5	100	211
20.0	6.6	11.2	36	99
18.0	6.8	10.8	34	92
14.3	2.6	5.0	5	20
11.6	0	0	0	0

The data for these experiments are recorded in Table 2 and are graphically illustrated in Figure 2. The minimum temperature for infection for the time employed lies between 11.6° and 14.3° C., the maximum between 32° and 34.4°, and the optimum between 23° and 29°.

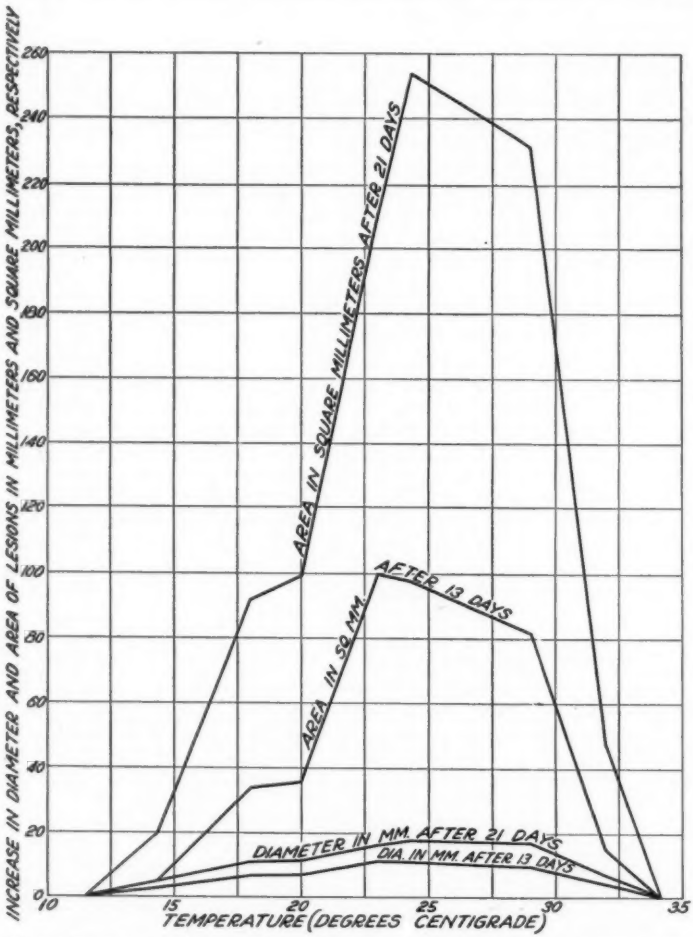

FIG. 2.—Curves representing the enlargement of lesions caused by *Ceratostomella fimbriatum* after 13 and 21 days

The effect of the passage of time was to enlarge the scope of the decay, except at 11.6° and 34.4° C., where there was none. More time would have allowed the limits of the infected areas to widen slightly. The difference in the amount of decay after the two periods of time (13 and 21 days) becomes progressively larger as the temperatures change from the maximum and minimum to the optimum. This relation is well illustrated by the area curves.

The diameter of fungus colonies and of fungus lesions have been used by other writers as a measure of the growth phenomena involved in the two cases. Although this standard affords a means of comparing the effect of different temperatures, it does not show the true relation of temperature either to the growth of the fungus or the decay involved in the enlargement of lesions of this type. The area of the lesions comes more nearly representing the actual quantity of decay (where the latter is a surface phenomenon) at a given temperature, and therefore the relation of decay to the different temperatures. The difference between the diameter curves and the area curves is striking (fig. 2); so striking, in fact, that one sees no good reason for using diameter as a measure of the amount of decay.

In the preceding experiments a comparison was made of the large lesions only that developed at the different temperatures. It seemed probable that there would be considerable variation in the rate of development in the various lesions, and consequently that a different relation might be found if the total number of lesions that developed at the various temperatures were used as a basis of comparison.

TABLE 3.—*Influence of temperature on infection of the sweet potato by black rot and on enlargement of the lesions after 11 days*

Psychrometer readings	Depression of wet bulb	Average area of lesions	Number of lesions
° *C.*	° *C.*	*Sq. mm.*	
36.0–34.6	1.4	0	0
33.0–31.8	1.2	0	0
31.5–28.9	2.6	16	3
29.5–27.8	1.7	31	32
27.0–25.0	2.0	73	35
24.0–23.0	1.0	58	74
23.0–22.0	1.0	49	163
20.0–19.1	0.9	27	165
18.5–18.0	.5	20	152
14.0–13.6	.4	7	22
11.8–11.0	.8	0	0
9.5– 9.3	.2	0	0
8.0– 7.8	.2	0	0

Table 3 gives the results of an experiment where 20 potatoes were inoculated in a spore suspension and placed at each of several temperatures. After 11 days all the lesions that had developed were measured. It will be noted that the number of lesions is somewhat smaller at the border temperatures than at the intervening temperatures. How greatly this fact influences the total area of lesions at a given temperature can not be stated with certainty, but judging from the curves obtained it seems improbable that the effect is large (fig. 3). There seems to be some correlation between the humidity of the air and the number of lesions, although the difference in the number of lesions at 24° and 23° C. is rather large, notwithstanding the fact that the depression of the wet bulb is the same at both temperatures.

A smoother curve (fig. 3) was obtained by this method than by that previously used. It is believed that this curve shows more accurately the decay present at the various temperatures than do the area curves in Figure 2, because it represents all of the lesions rather than a selected few. No breaks occur in it, and the cardinal temperatures are more definite. The results shown in Figure 3 were confirmed by another experiment which was run for 13 days.

INFLUENCE OF TIME ON THE DEVELOPMENT OF THE DISEASE

Some data were presented in Table 2 to show the correlation of time and size of lesions at various temperatures. These data permit of a comparison of the combined process of infection and enlargement of the lesions at one temperature with that at another temperature, but do not permit of a comparison of the enlargement of the lesions alone at different temperatures. The data in Table 4 show the influence of temperature after different periods of time on the enlargement of lesions as measured by their increase in area.

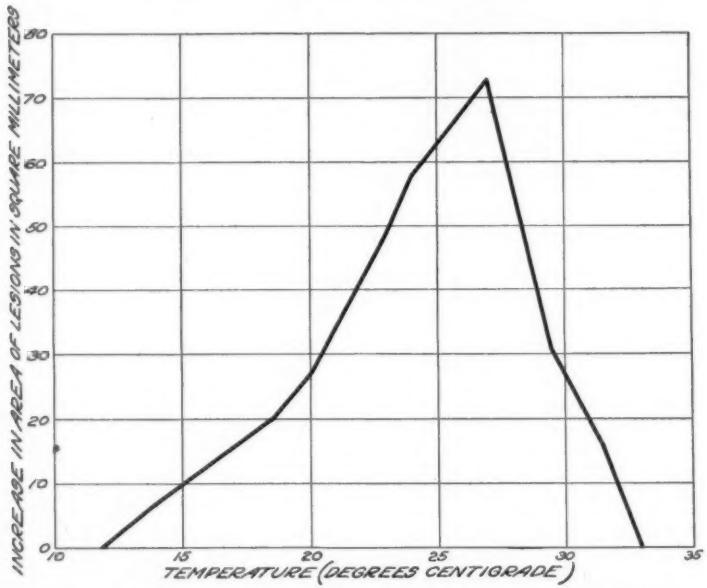

FIG. 3—Influence of temperature on development of black rot in the roots of sweet potatoes

TABLE 4.—*Influence of temperature on the enlargement of selected lesions in sweet potatoes after different periods of time*

Temperature	Average area of lesions in square millimeters after—						
	8 days	11 days	14 days	18 days	22.5 days	28 days	64 days
° C.							
33.0	12	23	23	24	27	32	----
31.0	12	26	32	37	46	------	----
28.0	11	27	32	42	60	109	----
25.0	11	24	31	40	52	119	----
23.0	13	29	39	51	69	115	----
20.0	11	20	27	35	49	69	----
18.5	12	25	35	49	60	139	----
14.0	12	22	26	30	36	60	----
12.0	13	18	19	21	26	29	----
9.5	11	15	20	------	23	------	----
6.0	13	16	16	------	19	------	20

The data were derived from two experiments and the averages were computed from 15 lesions at each temperature, except after 28 and 64 days, where the averages were computed from 7 lesions at each temperature and were obtained from one experiment. The potatoes were inoculated by being dipped in a water spore suspension. They were then placed at a temperature of 23° C. for a period of 5 days to permit infection to take place. The lesions were then measured and numbered, so that they could be recognized for subsequent measure-

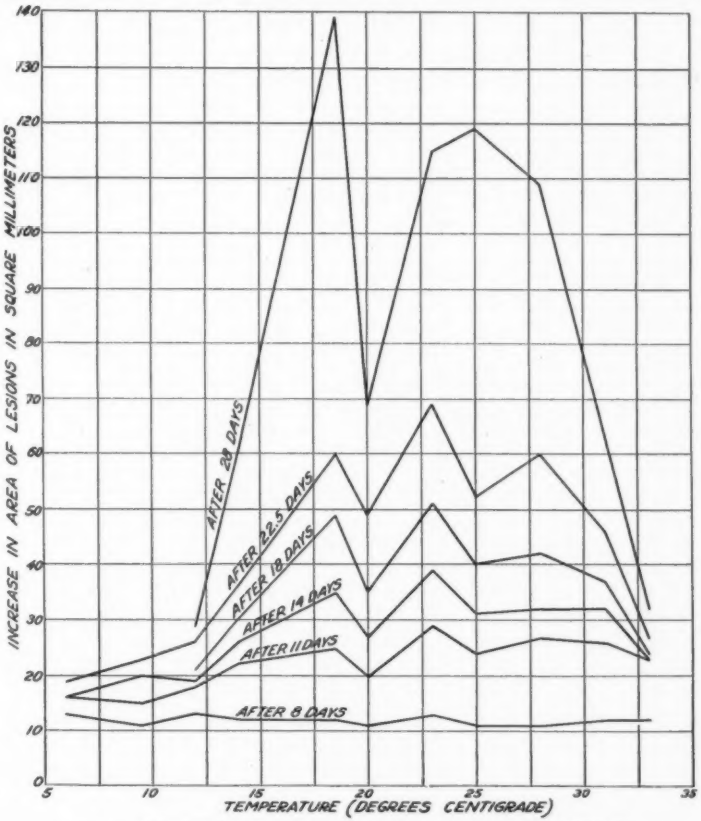

FIG. 4.—Enlargement of lesions in sweet potatoes after different periods of time

ments at different intervals of time. As soon as this was done the potatoes were divided into lots and distributed to the various temperatures in such a way that the sizes of the lesions were fairly comparable at the different temperatures. It will be seen by examining the curve labeled "After 8 days" in Figure 4 that the line is fairly straight. There are some variations in it, most of which persist throughout the successive periods. This fact indicates the difficulty of comparing directly the process involved in the enlargement where the areas of action are not identical from the beginning. These

variations are in some instances overcome at the more favorable temperatures and in some instances accentuated. With the exceptions noted, the curves (fig. 4) plotted from the data obtained in these experiments generally tend toward the normal curve with the lapse of time. These exceptions may be partially explained, at least. An examination of the notes from which these data were derived reveals a considerable variation in the rate of growth of the different lesions at a given temperature, and in some instances shows that the lesions stop enlarging altogether at different stages of development. These factors, especially the latter, are shown to be correlated with the variations in the curves. If the curves had been plotted from a larger number of lesions, these variations probably would not have been so evident.

There was some enlargement at all the temperatures employed (Table 4). It was not large at 33° and 12° C. and below. The enlargement at these extreme temperatures will be discussed more fully under the heading "Temperature limits of infection."

INFLUENCE OF TEMPERATURE AND TIME ON THE NUMBER OF INFECTIONS

The data recorded in Table 3 showed that temperature was a factor in determining the number of infections which occurred during the time employed (11 days). The number of infections rose from zero at 11.8° C. to 165 at 20°, but dropped off rapidly as the temperature rose from 23° to 31.5°, where there were only three infections; at 33° there were none. Humidity may have influenced this drop in the number of infections, for there was a general decrease in the relative humidity with the rise in temperature above 24°. However, this factor does not account for the difference in the number of infections at 23° and 24°, where the humidity was identical. Unpublished data show that there is a narrow range of humidity just below saturation, at a temperature of 23°, within which the number of infections is little affected by the difference in humidity, but that the number of infections drops rapidly after the humidity falls below this range. It can not be said definitely that this relation holds at other temperatures, but it would seem likely, and it is possible that the limit of this range of humidities was reached at 27° and 31.5° (Table 3).

TABLE 5.—*Influence of temperature on the number of lesions that develop after 21 days*

Psychrometer readings		Relative humidity	Number of sweet potatoes	Number of lesions per potato
Temperature	Depression of wet bulb			
° *C.*	° *C.*			
33.4	----	----	18	0.3
30.8	1.2	92	24	9.5
28.2	1.2	92	19	14.3
23.6	.9	93	24	19.4
23.0	1.0	92	20	16.1
19.9	1.4	88	24	13.4
18.3	.9	92	24	11.8
14.3	.8	91	18	21.0
12.0	.3	97	16	.06
9.0	.9	90	17	.0

The data recorded in Table 5 were obtained from an experiment designed to determine whether or not temperature governs the number of infections to the same degree if a longer period of time is em-

ployed. The results showed that the border temperatures limit the number of infections, but the increase in the number of infections from the minimum to 23.6° C. (near the optimum) was much smaller, and the maximum number of infections was obtained at 14°, which is several degrees below the optimum temperature for the development of decay. This exception would seem to be due to experimental error. One might account for the small difference in the number of infections in the same way at 18.3°, 19.9°, 23°, and 23.6° were it not for the direct correlation which exists between the number of infections and the rise in temperature within this range. An inverse correlation is also apparent between the number of infections and the depression of the wet bulb, the depression of the wet bulb decreasing from 1.4° at 19.9° to 0.9° at 23.6°.

Thus humidity may partially explain the difference in the number of infections at these temperatures. In any case, it is believed safe to conclude from the data presented that temperature as a determining factor in the number of infections becomes less important with the lapse of time, except at the border temperatures. This conclusion is confirmed by earlier work.[7]

TABLE 6.—*Temperature limits at which Ceratostomella fimbriatum infects sweet potato*

Psychrometer readings		Inoculated sweet potatoes						Remarks	Checks		Duration of experiment
Temperature	Depression of wet bulb	Sweet potatoes used	Sweet potatoes infected	Total lesions	Diameter of lesions	Isolations made	Organisms isolated		Sweet potatoes used	Sweet potatoes infected	
°C.	°C.	No.	No.	No.	Mm.	No.			No.	No.	Days
33.4	1.3	16	4	5		0					21
34.7	1.3	45	0	0	0	8	Fusarium sp.	8 doubtful lesions.			62
11.7	.5	16	16	153	3 to 18	0					62
11.0	.6	31	30	240	3 to 13	0					62
9.7	.6	56	8	1	4			8 doubtful lesions.			62
13.4	.4	14	13	78	3 to 15	0			13	0	56
11.1	.2	17	8	26	3 to 10	0			10	0	56
9.1	.2	11	0	0	0	0			9	0	56
13.2	1.1	15	3	12		0			15	0	103
11.4	.8	16	2	3		3	Ceratostomella fimbriatum.				103
9.5	.6	14	5	10		5	3 Fusarium sp.; 1 Alternaria sp; 1 Penicillium sp.				103
13.2	1.0	18	17	99	5 to 50	0			18	0	108
11.5	.9	17	16	101	5 to 20	6	Ceratostomella fimbriatum.				108
9.5	.6	18	2	2	5 to 8	4	2 Fusarium sp.; 2 Penicillium sp.	1 doubtful lesion.			108

TEMPERATURE LIMITS OF INFECTION

The data recorded in Table 6 were obtained from three experiments, which had for their purpose the determination of the upper and lower temperature limits for infection. The potatoes were inoculated by dipping in a spore suspension. In one of the experiments isolations were made from the lesions that developed at the border temperatures. This procedure was adopted because in some instances the lesions were not typical. Checks were run in some cases to make sure that the infections were due to the inoculum. The check potatoes were dipped in tap water instead of a spore suspension.

[7] AMES, A. THE TEMPERATURE RELATIONS OF SOME FUNGI CAUSING STORAGE ROTS. Phytopathology 5: 11–19. 1915.

Five typical black-rot lesions developed at a temperature of 33.4° C. in 16 days. This temperature is almost as high as the highest temperature (34.5°) at which *Ceratostomella fimbriatum* grew on sweet-potato agar (Table 1). There were no infections at 34.7°, even at the end of 62 days. It would seem, therefore, that the maximum temperature for infection was between 33.4° and 34.7° C.

The lowest temperature at which infection occurred was 9.5° C. This conclusion is based entirely on the character of the lesions, which, however, seemed to be typical. *Ceratostomella fimbriatum* was not obtained in any of the isolations made at this temperature. It would seem that if any infection did occur the pathogene either died out or gave way to other fungi. At any rate the infection that occurs at this temperature is of little consequence. This contention is further supported by the fact that no growth of the pathogene, except in one doubtful case, occurred at 9.5° C. in 55 days and very slight growth occurred at 10° in 73 days. No infection occurred at 9.1° in 56 days. Infection took place fairly readily at 11° and the pathogene was recovered at 11.4°. It would seem from these results that the lower temperature limit for infection lies between 9° and 11°.

These results indicate that sweet potatoes can be stored at temperatures from 9° to 10° C. without much danger of infection, although the pathogene may be present. A humidity below 90 per cent would further insure against infection.

If infection has already occurred some enlargement of the lesions may be expected at 9.5° C. (Table 4) and possibly at even a lower temperature, but it would be too slight to be of any consequence. Even at 12° the enlargement of lesions is rather slow.

The slight enlargement of the lesions at temperatures below the minimum may have been influenced by the removal of the potatoes to the laboratory (the temperature of the laboratory ranged from 20° to 23° C.) during the periods in which the measurements were made. The time consumed by these measurements was never more than a half hour, except when the first measurements were made; hence it is believed that this change of condition had little effect on the enlargement of the lesions.

The temperatures, therefore, that recommend themselves for the storage of sweet potatoes where black rot is a factor are those from 10° to 12° C., since sweet potatoes can not be stored below these temperatures without becoming infected with *Mucor racemosus*.[8] It is believed possible to practically eliminate the spread and development of black rot if the potatoes are stored at these temperatures and at a relative humidity below 90 per cent.

DISCUSSION AND CONCLUSIONS

Ceratostomella fimbriatum will grow on sweet-potato agar over a range of temperatures extending from 9.5° to 34.5° C. (Table 1). This range corresponds closely to that obtained for infection. However, infection was doubtful at the two extreme temperatures of this range. Growth of the pathogene, as represented by the area of the colonies, increases very rapidly as the temperature rises from the minimum (9° to 10° C.) to 27° after which it declines with the rise in temperature until 36° is reached, at which point all growth ceases.

[8] LAURITZEN, J. I., and HARTER, L. L. SPECIES OF RHIZOPUS RESPONSIBLE FOR THE DECAY OF SWEET POTATOES IN THE STORAGE HOUSE AND AT DIFFERENT TEMPERATURES IN INFECTION CHAMBERS. Jour. Agr. Research 24: 441–456, illus. 1923.

This rate of growth is due not only to temperature but to the area of the medium to which it is exposed, which progressively increases with the increase in diameter of the lesions.[9]

Infection may occur through the normal skin, but usually there is evidence of tissue disturbances at the point of infection in the form of raised areas, slight abrasions, and small rootlets. Wounding is not necessary in order for infection to occur, although there is evidence that wounding will aggravate infection and the development of the disease.

The increase in the size of the lesions with the lapse of time at any of the temperatures between the maximum and the minimum is not as rapid as the enlargement of the colonies of the pathogene on sweet-potato agar. This fact would seem to indicate that either sweet-potato agar is the more favorable medium for the development of the fungus or that there is some inhibiting factor retarding the enlargement of the lesions.

The fact that the lesions enlarge at temperatures below the minimum for infection (Table 4) while the fungus does not grow at these temperatures may raise some question as to the foregoing conclusion, at least under the latter conditions indicated. What would happen if growing colonies of the fungus on artificial media were exposed to these temperatures is not known, but it seems probable that the colonies would enlarge as did the lesions. It has been observed that new transfers of fungi do not exhibit the normal rate of growth at the start. To make the above conclusion valid more experimentation is required.

The rate of enlargement as represented by the area of the lesions increases rapidly as the temperature rises above the minimum (9° to 10° C.) to temperatures of 23° and 27° C., above which there is a decline in the rate as the temperature is raised to 34.7°, where there is no infection.

A part of this increase [10] in the rate of enlargement of the lesions with the rise in temperature is due to the increase in the area exposed to the action of the fungus with the increase in the diameter of the lesions. This increased area of action of the fungus as the temperature is raised to the optimum becomes progressively greater with the lapse of time, thus enhancing the enlargement of the lesions.

Taking into consideration the methods employed in measuring the influence of temperature on the development of the disease, it may be concluded that the optimum ranges from 23° to 27° C. This variation is not large when it is considered that three methods of procedure and several experiments were employed. Even where the same method was used in two different experiments some variation occurred. This was shown by the fact that in an experiment conducted in the same fashion as the one from which the data recorded in Table 3 were obtained the optimum was 24.5°, while the optimum recorded in Table 3 is 27°. It is believed that the optimum for the growth of a fungus, for infection, or for the development of the disease can never be assigned to a particular degree of temperature. The variation in the fungus and the host, even though the conditions surrounding them may be constant, is such as to make the optimum limited to a particular temperature improbable except in a single experiment.

[9] LAURITZEN, J. I., and HARTER, L. L. THE INFLUENCE OF TEMPERATURE ON THE INFECTION AND DECAY OF SWEET POTATOES BY DIFFERENT SPECIES OF RHIZOPUS. Jour. Agr. Research 30: 793-810, illus. 1925.

[10] LAURITZEN, J. I., and HARTER, L. L. Op. cit.

The range of temperature at which black rot will develop is wider than that at which infection will occur. Infection has taken place at temperatures of 9.5° and 33.4° C. (Table 6), while there has been an enlargement of lesions at temperatures from 6° to 33.4° (Tables 4 and 6). This fact has a practical bearing. Potatoes that have not been infected when they are placed in storage, although the pathogene is present, can be kept at temperatures of 10° to 12° without much danger of infection, especially if the relative humidity is below 90 per cent. If infection has taken place, the lesion will continue to develop (Table 4, fig. 4). Fortunately the rate of enlargement is very slow, hence the disease can be held in check. Besides there is some evidence that the fungus dies out at a temperature of 9.5° (Table 6). Since potatoes that are infected are not regarded as edible, the holding of the disease in check loses some of its significance. Temperature governs the number of lesions during the early stages of infection (Table 3), but as the time is extended from 11 to 21 days its influence becomes strikingly less at temperatures between 14° and 28° C. (Table 5), where there is little difference in the number of infections. The number drops off below and above these temperatures.

SUMMARY

The temperature range for the growth of *Ceratostomella fimbriatum* on sweet-potato agar extends from 10° to 34.5° C.

The optimum, maximum, and minimum temperatures for the growth of the fungus are 23° to 28.5° C., 34.5° to 36°, and 9° to 10°, respectively.

The range of temperatures at which *Ceratostomella fimbriatum* will infect sweet potatoes extends from 9.5° to 34.5° C., corresponding closely to that of the growth of the pathogene. The fungus seems to die out at a temperature of 9.5°.

The optimum, maximum, and minimum temperatures for infection and the development of the disease are: Optimum, 23° to 27° C.; maximum, 34.5° to 36°; and minimum, 9° to 10°.

The number of infections that will occur between 9° and 11° C. is small.

The temperature range at which it has been shown that black-rot lesions will enlarge is from 6° to 33.5° C.

The rate of enlargement is slow between 6° and 14° C. and increases very rapidly above 14° until it reaches a maximum between 23° and 27°. It varies greatly at any given temperature and in some instances ceases altogether.

The number of infections is governed by the temperature which prevails (Table 3) during the early stages of infection (11 days). The number of infections increased from 22 at 14° C., the minimum for this period, to a maximum at temperatures near the optimum for the development of the disease (20° and 23°). With a further rise in temperature the number of infections decreased to three at 31.5° and at 33° ceased altogether.

As the experimental period was extended from 11 to 21 days (Table 5) the influence of temperature on the number of infections became less apparent, except at the border temperatures. The differences in the number of infections at temperatures of 14°, 18°, 20°, 23°, 23.6° and 28° are relatively small.

FRUIT-BUD DIFFERENTIATION AND SUBSEQUENT DEVELOPMENT OF THE FLOWERS IN THE HICORIA PECAN[1]

By J. G. WOODROOF, *Assistant Horticulturist*, and NAOMI CHAPMAN WOODROOF, *Assistant Botanist, Georgia Agricultural Experiment Station*[2]

INTRODUCTION

A correct understanding of the time of fruit-bud differentiation and some of the related activities of the pecan should have an important bearing upon cultural practices. More efficient methods of fertilizing and pruning pecan trees might be evolved from accumulated information of this kind.

The purpose of the present study is to determine the time at which flowers are differentiated and to trace their development to the time of pollination. The question resolves itself into a study of the changes that take place in bud development, the time when these changes occur, and the conditions that influence them.

Benson and Welsford [3] described the morphology of the ovule and female flowers of *Juglans regia*, with special reference to the vascular system. Holm [4] described the staminate flower of *Carya alba* and of *Juglans nigra*. Stuckey [5] studied certain phases in the development of the pecan flowers. He recorded, for more than 25 varieties of *Hicoria pecan*, the dates on which the staminate flowers reach maturity and shed their pollen and the dates on which the pistillate flowers become receptive.

The senior author,[6] in a study of the development of the staminate flowers of 28 varieties of pecan, found that catkins are differentiated in lateral buds on new shoots in late spring. They pass the winter as dormant buds and complete their development the following spring.

METHODS

Terminal buds of the Alley and Frotscher varieties were collected every other day, beginning March 13, 1925, and continuing until the pistillate flowers were separable as individual flowers on April 7 and 11, respectively. Flowers were then collected every second day until pollination time. The following season buds were collected at 20-day intervals from November until March. The Stuart and Jerome varieties were extensively used in detailed studies of the flower at pollination time.

Material was fixed in both Flemming's weaker solution [7] and Juel's fixative.[8] Flemming's solution rendered the material too brittle to

[1] Received for publication Apr. 20, 1926; issued October, 1926.

[2] The writers are indebted to B. B. Higgins of this station for constructive criticisms of the technic employed in the investigations herein reported.

[3] BENSON, M., and WELSFORD, E. J. THE MORPHOLOGY OF THE OVULE AND FEMALE FLOWER OF JUGLANS REGIA AND OF A FEW ALLIED GENERA. Ann. Bot. [London] 23: 623–633, illus. 1909.

[4] HOLM, T. MORPHOLOGICAL STUDY OF CARYA ALBA AND JUGLANS NIGRA. Bot. Gaz. 72: 375–389, illus. 1921.

[5] STUCKEY, H. P. THE TWO GROUPS OF VARIETIES OF THE HICORIA PECAN AND THEIR RELATION TO SELF-STERILITY. Ga. Agr. Expt. Sta. Bul. 124: 127–148, illus. 1916.

[6] WOODROOF, J. G. THE DEVELOPMENT OF PECAN BUDS AND THE QUANTITATIVE PRODUCTION OF POLLEN. Ga. Agr. Expt. Sta. Bul. 144: 134–161, illus. 1924.

[7] CHAMBERLAIN, C. J. METHODS IN PLANT HISTOLOGY. Ed. 3, rev. 314 p., illus. Chicago, Ill. [1915.]

[8] Formula for Juel's fixative: $ZnCl_2$, 2 gms; acetic acid, 2 c. c.; 95 per cent alcohol, 50 c. c.; distilled water, 50 c. c.

section well. Juel's fixative proved more satisfactory and was used for most of the work. The buds were somewhat difficult to manage owing to the heavy growth of hairs on the young leaves. It was necessary to trim them very closely so as to remove as many as possible of the young leaves.

All material was embedded in paraffin and the sections stained in Haidenhain's iron-alum haematoxylin.

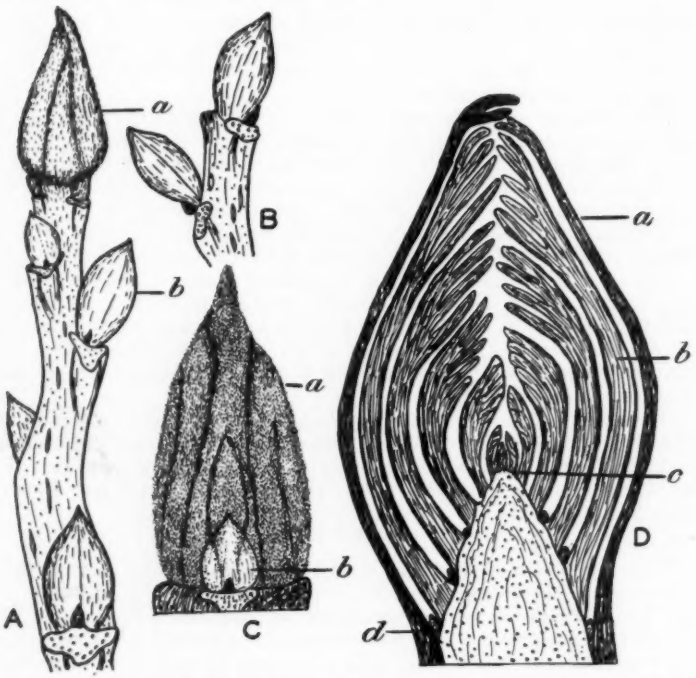

FIG. 1.—Types and position of buds. A, shoot bearing terminal (*a*) and lateral (*b*) buds; B, shoot with only lateral buds; C, terminal bud after the scales are shed (*a*) young leaf, (*b*) lateral bud; D, internal structure of terminal bud; (*a*) terminal bud scale; (*b*) inclosed leaves; (*c*) point of differentiation; (*d*) lateral bud

DIFFERENTIATION IN THE TERMINAL BUDS

The pistillate flowers are normally differentiated from the terminal buds of the dormant season, but when a terminal bud is not present one or more lateral buds may differentiate pistillate flowers.

BUDS

In the absence of a terminal bud the casual observer will often mistake the apical, dormant, lateral bud for a terminal bud, since the scar left by the shedding of the nut cluster of the previous season is very small and inconspicuous. The color of the two buds is similar, but the terminal buds (fig. 1, A, *a*) are angular, uneven, and somewhat larger than the dormant lateral buds (fig. 1, A, *b*).

The terminal buds do not have the same type of scales as the lateral buds, but are protected by the thick, flattened, outer leaves, which are practically identical with the inner dormant leaves of the bud. Several of these leaflike scales are shed as the bud swells and only the innermost develop into leaves. In the axil of each scale is a very small lateral bud (fig. 1, D, *d*).

LEAVES

A terminal bud in late winter contains the full number of leaves that will develop the following season. This number is definitely determined when the first pistillate flower appears. The larger leaves grow over and around the smaller and more recently formed ones (fig. 1, D). In the axil of each scale and leaf is a developing lateral bud of the following season. (Fig. 1, D, *d*.) The young leaves are light green in color, covered by a thick growth of silvery hair.

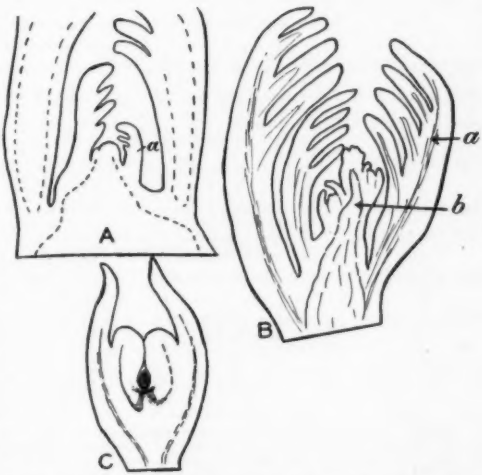

FIG. 2.—Pistillate flower differentiation. A, growing point of a terminal bud during the winter; B, growing point on March 13, after several pistillate flowers (*b*) have been differentiated; *a*, subtending leaves; C, young basal flowers on date when first flower clusters were visible above the leaves. Terminal drops on May 1 have reached a similar stage of development. All × 9

FLOWERS

Woodroof[9] concluded that the internal character of the buds does not materially change from the time of harvest in November until the middle of February (fig. 2, A), and that differentiation of the first pistillate flowers occurs during the last 10 days of February and the first week in March. By the second week in March some of the flowers are becoming quite large and the floral characters are beginning to appear (Table 1). The basal flowers appear first, followed by others placed alternately along the elongating floral axis (fig. 2, B and C). Several stages of development may be seen in a differentiating cluster of flowers (fig. 2), and this is true even when most of the flowers of the cluster are ready to be pollinated. The basal flowers show some of the floral characters while the apical ones are mere papillae and others have not been differentiated. Simultaneously with the differentiation and early development of the flowers, the scales are shed and the leaves begin to unfold.

Where the terminal bud has been present, but was destroyed before the time of differentiation (from February 15 to March 1), lateral

[9] WOODROOF, J. G. THE DEVELOPMENT OF PECAN BUDS AND THE QUANTITATIVE PRODUCTION OF POLLEN. Ga. Agr. Expt. Sta. Bul. 144: 134–161, illus. 1924.

buds are "forced" to differentiate pistillate flowers; but if destroyed later than March 15–20 there will be no pistillate flowers to reach the receptive stage, and a crop failure for that year will result. In pecan trees which bear an annual crop of nuts a large percentage of the clusters of pistillate flowers must come from twigs produced from lateral buds (fig. 3). Theoretically, the pecan is strictly an alternate year bearer; while practically it may bear annual crops because the years in which shoots from terminal buds produce nuts, shoots from lateral buds do not bear, but produce terminal buds in preparation for a crop the following year.

TABLE 1.—*Stages of development in the buds and flowers of the pecan and the dates on which they occur*

Date	Pistillate flowers or buds	Staminate flowers or buds	Vegetative growth [a]
Feb. 15 to Mar. 1	Differentiation in terminal buds.	Outer bud scale bursting	Very little.
Mar. 11 to Mar. 15	Bud scales bursting; differentiation continued.	Shedding of outer bud scale	Do.
Mar. 15 to Apr. 1	Differentiation of apical flowers; development of basal flowers.	Outer scale shedding completed.	Very young leaves exposed.
Apr. 1 to Apr. 15	Rapid development; may appear visible above leaves.	Inner bud scale bursting	Exposed leaves growing rapidly.
Apr. 15 to May 1	Flowers reach full size; may become receptive.	Catkins reach full size; may shed pollen.	Very rapid.
May 1 to May 15	Pollination completed; stigmas dry; first "drop."	Pollen shed; catkins drop; flowers may be differentiated in new buds.	Do.
May 15 to June 1	Rapid growth of nutlets	Rapid development of lateral buds; differentiation of flowers.	Do.
June 1 to Oct. 1	Continued growth and development of nuts.	Continued development of lateral buds.	Continual growth.
Oct. 1 to Nov. 1	Nuts mature	Lateral bud growth decreases.	Terminal bud may form. [b]
Nov. 1 to Feb. 1	None	Very slow growth in lateral buds.	Almost inactive.

[a] Though the formation and development of leaves has not been systematically studied, careful observation has shown that differentiation is active in the vegetative portion of the lateral buds as well as in the terminal bud during the late fall and winter. In the lateral buds the process continues throughout the spring and summer into the fall and winter until the terminal bud differentiates pistillate flowers in February of the next year. Leaf differentiation ceases on a particular shoot when pistillate flower differentiation begins.

[b] If the shoot was terminated with a cluster of nuts during the current year no terminal bud will be formed. A scar will mark the place of attachment of the nut cluster. Almost immediately upon the formation of the leaf a lateral bud forms in the axil, in which is differentiated staminate flowers. Thus staminate flower formation continues over almost the entire growing season.

EARLY DEVELOPMENT OF THE PISTILLATE FLOWERS

The four bracts of the calyx are the first parts of the flower to appear, and these are followed by the primordia of the stigmas. Early in the development of the bracts and the stigmas the traces of a vascular system may be seen in the longitudinal sections of the flower (fig. 2). The stigmas soon inclose the ovarian cavity. An ovule begins to develop in the basal flower about April 15, the approximate date on which the young flower cluster is first visible above the unfolding leaves (fig. 2, C). A week or 10 days after the appearance of the ovule an integument begins to develop.

THE PISTILLATE FLOWER CLUSTER AT POLLINATION TIME

The pistillate flowers develop very rapidly, and are receptive about two months after the differentiation of the basal flowers. The flowers are borne sessilely along a zigzag central axis. There are

from 4 to 20 or more flowers in a single spike. The number of flowers per spike, or flower cluster, is a varietal character. In 1925 about 90 per cent of the Teche and Alley varieties bore 4 receptive flowers per cluster, with a few of 3 and 5 flowers and none with 6. Most of the clusters of the Nelson and Mobile varieties bore 6 receptive flowers per cluster, with a few of 5 and 7, but none with 3 and 4. With the Stuart and Frotscher varieties, 5 was the commonest number.

FIG. 3.—The cycle of terminal bud formation: *a*, cluster of nuts produced in "even" years; *b*, terminal bud produced in "odd" years

"DROPS"

Regardless of the number of flowers receptive per cluster at the time of pollination, there are always some undeveloped flowers at the apex of each cluster. (Fig. 4, *a*.) There may be from three to half a dozen of these which differentiate very late but grow and develop normally until the time of pollination, after which they begin to drop without becoming receptive. This first "drop" leaves a scar at the terminus. In no case has a cluster of nuts been observed which had developed to its fullest capacity without showing the small scar indicating the "dropping" of the terminal flowers. Longitudinal sections of these undeveloped flowers show them to be normal, but not so far advanced in development as the other flowers of the cluster. (Fig. 2, C.) In other words, all flowers which are not receptive at the time of pollination are soon shed from the cluster. This first "drop" means that from 20 to 40 and sometimes 50 per cent of the differentiated flowers of a spike are shed at pollination time.

FIG. 4.—A cluster of pistillate flowers. *a*, Terminal flowers of the cluster which have never been found to develop; *b*, a flower which develops only under the most favorable conditions, otherwise it "drops;" *c*, a flower which develops into a nut

INDIVIDUAL FLOWERS AT POLLINATION TIME

EXTERNAL CHARACTERS

An individual flower measures from 5.5 mm. to 8 mm. in length, from 3 mm. to 4.5 mm. of which are inclosed in a light green pubescent calyx; the remainder is stigma. The calyx is marked by four ridges, giving it a four-sided appearance and dividing it into four sections, each of which is terminated by a tapering bract from 3

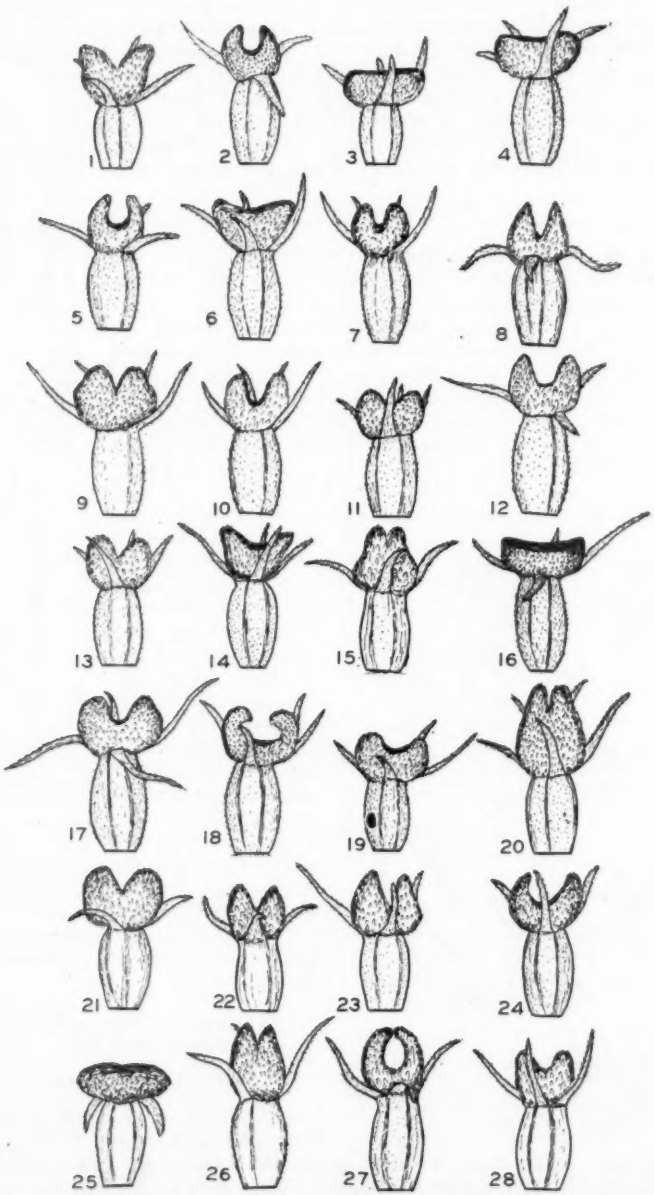

FIG. 5.—Pistillate flowers of 28 varieties of pecans, showing relative size, shape, and curvature of the stigmas, length and curvature of the bracts, and relative size of the entire flower. × 4. (See Table 2 for varieties and colors of stigmas to correspond with the numbers indicated)

mm. to 5 mm. in length, according to the variety. These may be erect, extend laterally, or be reflexed downward along the sides of the flower (fig. 5). Receptive stigmas are sessile, variously shaped, and colored, and a viscid fluid covers the uneven surfaces. The differences among varieties in the size, color, and shape of the stigmas are varietal characters and are very distinct. The writers have not been able to relate any of these floral characters with later characters that appear in the mature nut.

FIG. 6.—Longitudinal section of flower at time of pollination in the plane of the middle septum. O, ovule; I, integument; Se. B, septum bundles; B, outer series of vascular bundles; B', inner series of bundles. × 9

TABLE 2.—*Name and color* [a] *of stigmas of each of the 28 varieties of pecan illustrated in Figure 5, April 29, 1925*

No.	Variety	Color	No.	Variety	Color
1	Alley	Vivid green.	15	Nelson	Vivid green.
2	Appomattox	Do.	16	Pabst	Do.
3	Atlanta	Night green.	17	Randal	Ox-blood red.
4	Beverage	Vivid green.	18	Russell No. 2	Brick red.
5	Bradley	Bottle green.	19	Rome	Dull dark purple.
6	Centennial	Ox-blood red.	20	Russell	Night green.
7	Curtis	Vivid green.	21	San Saba	Warbler green.
8	Delmas	Night green.	22	Schley	Vivid green.
9	Frotscher	Vivid green.	23	Stuart	Do.
10	Jerome	Dull dark purple.	24	Success	Ox-blood red.
11	Mantura	Vivid green.	25	Teche	Night green.
12	Mobile	Ox-blood red.	26	Unknown	Do.
13	Moneymaker	Vivid green.	27	Van Deman	Do.
14	Moore	Bottle green.	28	Waukenah	Olive green.

[a] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

INTERNAL CHARACTERS

The vegetative regions of the flower resemble those of the young growing nut. The vascular system is fairly complete and serves as a means of locating and distinguishing the different regions, which are in some cases rather indistinct.

The outer is the calyx or hull region and is composed of loosely arranged, irregularly shaped cells. A series of fibro-vascular bundles extend into this region from the main axis of the spike (fig. 6, B). The second, or shell region, is composed of small uniformly shaped, compactly arranged cells. Two opposite suture bundles are located

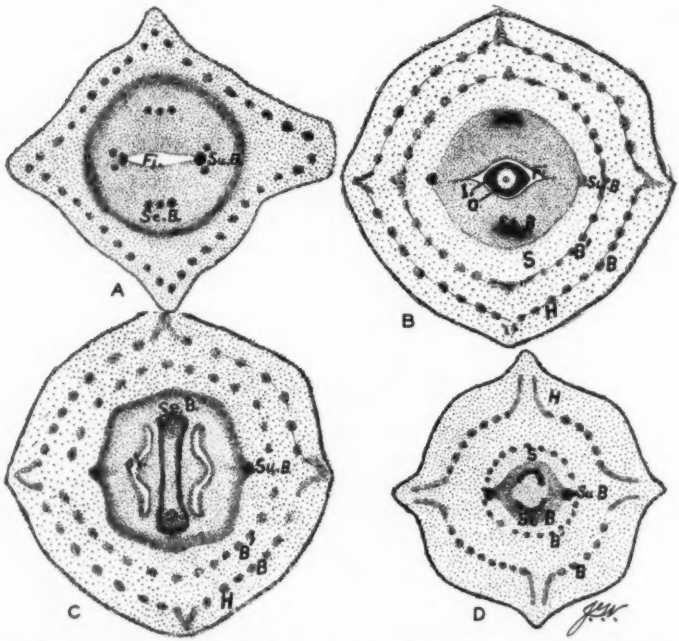

FIG. 7.—Pistillate flower at time of pollination. A, cross section near the base of bracts; B, cross section through the ovule and integument; C, cross section just below the attachment of ovule; D, cross section where the inner series of bundles enter the shell region. H, region of the hull; B, outer series of vascular bundles; B′, inner series of bundles; S, region of the shell; Su. B, suture bundles; Se. B, septum bundles; Fi, fissure in packing tissue; I, integument; O, ovule. × 9½

in this region at right angles to the plane of the stigmas. These extend upward from the base to the apex of the shell region. Near the apex each separates into three distinct bundles (fig. 7, Su. B).

Between the regions of the shell and hull is a second series of fibrovascular bundles. This series is parallel to the first and is connected with it by lateral branches (fig. 6). The basal extremities of the second series are connected with the vascular system of the shell sutures and of the interior of the flower. The apical extremities extend into the stigma and appear in cross section as a semicircle. The inner series of bundles is not connected with the

outer series at either extremity but only by lateral branches, which are placed at irregular intervals along the sides of the flowers (fig. 6).

The ovary is imperfectly divided by a middle septum located in the plane of the stigmas. The ovule and integument are attached at the base of an opening near the apex of the septum, leaving them free to expand into a fissure (fig. 7, Fi), which is formed in a tissue which almost fills the ovary on either side of the septum. This has been called by Benson and Welsford "packing tissue." [10] It is this fissure which later enlarges to accommodate the expanding embryo (kernel) of the nut.

The ovule is about two-thirds inclosed by the integument when the flowers become receptive (fig. 6), and the vascular system of the septum has become quite distinct. There appears to be no definite communication between the septum bundles and those next the shell region, this not being established until after pollination has taken place. The vascular bundles of the septum develop independently from vascular traces; and later become connected with the series of bundles next the shell region as described by Benson and Welsford [11] for *Juglans regia*. Each of the two parallel bundles of the septum become divided near the apex into a semicircle of bundles (fig. 7). Developing fibrovascular bundles are distinguishable in cross sections of the integument.

The embryo sac is not mature at the time of pollination; consequently fertilization can not take place then. Four megaspores appear in nearly all of the ovules on that date, three of which have begun to disintegrate, and the nucleus of the mother cell has divided once and in some cases twice.

SUMMARY

Pistillate flowers in *Hicoria pecan* are differentiated in terminal buds from February 15 to March 1.

In the absence of a terminal bud pistillate flowers may be differentiated in lateral buds.

Pistillate flowers become receptive about two months after differentiation.

The first "drop" occurs at pollination time.

Pistillate flowers are not ready for fertilization at the time of pollination.

Staminate flowers are differentiated in lateral buds from 6 to 12 months before the pollen is shed.

[10] BENSON, M., and WELSFORD, E. J. THE MORPHOLOGY OF THE OVULE AND FEMALE FLOWER OF JUGLANS REGIA AND OF A FEW ALLIED GENERA. Ann. Bot. [London] 23: 623-633, illus. 1909.

[11] BENSON, M., and WELSFORD, E. J. OP. CIT.

THE BROWN-POCKET HEART ROT OF STONE-FRUIT TREES CAUSED BY TRAMETES SUBROSEA WEIR[1]

By S. M. ZELLER

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INTRODUCTION

The production of prunes and peaches is an important industry in the Pacific Coast States, and any factor which contributes to the lengthening of the productive life of these stone-fruit trees is worthy of consideration. The more the situation is studied the more it becomes apparent that wood decay in this region is the most serious factor which leads to early senility or decrepitude of producing prune and peach trees. The one predominating heart rot of these trees is that produced by *Trametes subrosea* Weir (*4*).[2] In a previous paper the writer (*6*) referred to this fungus as *T. carnea*. The disease of red cedar caused by this organism was described by Von Schrenk (*2, pp. 16–21*), but its occurrence and destructiveness as an orchard disease has never been described, to the present writer's knowledge.

DISTRIBUTION

In stone-fruit orchards *Trametes subrosea* is found from the coastal and interior valleys of central California northward to the coastal districts of British Columbia. Wherever prunes and peaches are grown in western Washington and western Oregon, this fungus is found doing a major part of the heart-rot damage. In French prune orchards in California where this disease has been serious, this fungus has been responsible for 50 or 60 per cent of the wood decay according to W. W. Thomas, a student of this disease in that State. As is true of all such wood-inhabiting fungi, this disease is most frequently found in orchards which have insufficient care.

Being a wound parasite, *Trametes subrosea* occurs in relation to unprotected infection courts, such as pruning cuts and broken branches. Figure 1 shows the fruiting bodies of the fungus near two unprotected pruning cuts on peach wood.

NATURE OF THE HEART ROT

As the brown cubical rot produced by this organism is more or less restricted to definite pockets in the wood, it has been called brown-pocket rot. Figure 2 shows the decayed pockets in prune wood, in both longitudinal and cross sections, and illustrates the type of this decay.

The individual pockets contain a brown, punky wood which crumbles easily and is usually shrunken and cracked into cubical form.

The changes which the fungus causes in the wood of peach does not differ from that produced in prune. Great chemical changes take

[1] Received for publication Feb. 15, 1926; issued, October, 1926.

[2] Reference is made by number (italic) to "Literature cited," p. 693.

place in the wood because of the digestive activity of the fungus, and it could be inferred a priori that the group of cytolytic enzymes which give other fungi the power to bring about brown rot must be active agents of *Trametes subrosea*. Nevertheless, the writer carried out some enzymic experiments with the mycelium of this organism grown on peach-wood sawdust, to supplement his observations on

FIG. 1.—Fruiting bodies of *Trametes subrosea* Weir near two unprotected pruning cuts which were improperly made. The host is peach

the glucoside-splitting and cellulose-hydrolyzing enzymes of *Lenzites saepiaria* (*5*). After a pure culture of *T. subrosea* had grown on the peach-wood sawdust for about nine months it was removed from the culture jars, dried, and ground. The sawdust was so thoroughly decayed that it was readily ground to a fine powder by rubbing between the palms of the hands. This fungous powder was used in the following experiments.

EMULSIN EXPERIMENT

To test for the presence of emulsin, a 1 per cent solution of amygdalin was used as a substrate, for this is perhaps the most prevalent glucoside in the wood of stone-fruit trees. Fifty cubic centimeter portions of the amygdalin solution were placed in each of two small flasks, and 2 gm. of the fungous powder were added to each. To each of two other flasks 2 gm. of moistened fungous powder were added, and they were autoclaved at 15 pounds for 5 minutes before the 50 c. c. of amygdalin solution were added. In another flask 50 c. c.

FIG. 2.—Longitudinal and transverse sections of a branch showing the brown-pocket rot in the heartwood. The host is prune

of the amygdalin were placed as a control. To all five flasks sufficient toluene was added for antisepsis. The flasks were corked and allowed to stand at room temperature for five days, after which the two flasks with fungous powder which had not been autoclaved gave a strong odor of benzaldehyde and a very definite Prussian-blue test for hydrocyanic acid. The plain control gave neither of these tests, but the autoclave control gave a slight test for hydrocyanic acid. This is accounted for, perhaps, by the presence of small quantities

of amygdalin in the peach wood used as the culture medium. Thus it was demonstrated that the mycelium of *Trametes subrosea* secretes emulsin as an active digestive ferment.

LIGNINASE EXPERIMENT

A quantity of the cubical brown rot from the pockets produced by *Trametes subrosea* in prune wood was gathered, powdered, and soaked in the least possible amount of chloroformed water for about 24 hours, after which the water was filtered after being expressed from the decayed wood. The enzymes were precipitated with 95 per cent alcohol, collected on filter paper, and dried. This enzymic precipitate was dispersed in a small quantity of water and used in the following experiments.

Very thin shavings of sound prune wood were placed in a flask and digested in a 1 per cent diastase solution for 16 hours. After this they were soaked in several rinsings of distilled water for three days, to remove as much of the soluble substances as possible, and then the shavings were dried.

One gram of these shavings was placed in each of three test tubes. To one was added 10 c. c. of the enzyme dispersion; to another, 10 c. c. of distilled water; and to the third, 10 c. c. of the enzyme dispersion which had been autoclaved at 15 pounds' pressure. Toluol was added to all three, and then they were corked and incubated at room temperature for 25 days. After this time the liquids were decanted from the three tubes and saved, and the shavings were boiled in absolute alcohol for 15 minutes. When the alcohol was tested with phloroglucin acidified with HCl, a deep red color was given in the first tube, but the second and third remained clear and colorless. This is Czapek's (*1*) test for hadromal, and is an indication that *Trametes subrosea* liberates the extracellular enzyme, ligninase, which has the power of splitting lignin. Czapek says that lignin is a cellulose-hadromal ether which may be split by an enzyme.

The aqueous solutions which were decanted from the shavings were tested with Fehling's solution. The first reduced Fehling's solution, while the second and third yielded no trace of copper. The results show that these reducing substances are due to enzyme action, and that they must be aldoses. These sugars may be formed from various substances. There is probably some tannin and amygdalin in prune wood. Besides this there is the hydrolysis of the cellulose to reducing sugars, as demonstrated by the following experiment which was conducted simultaneously with the above.

CELLULASE EXPERIMENT

Very small bits of filter paper were placed in three test tubes. To the first was added 10 c. c. of the enzyme dispersion; to the second, 10 c. c. of the enzyme dispersion which had been autoclaved; and to the third, 10 c. c. of distilled water. Toluol was added to all three, and they were corked and incubated for 25 days.

After this the liquid was decanted and tested with Fehlings' solution. The first tube gave a heavy precipitate of copper oxide, while there was not a trace in the second and third tubes. This indicated that cellulase was present as an active agent in the mycelium of *Trametes subrosea*.

HEMICELLULASE EXPERIMENT

Small chips of the paragalactan from the endosperm of date seeds were also used as a substrate in the same way as described above for prune-wood shavings and filter paper. In this case, when the first tube was tested with Fehling's solution a heavy precipitate of copper oxide was given, but none was evident in the control tubes. This demonstrates the presence of hemicellulase, an enzyme which splits the paragalactan, a hemicellulose, yielding a mixture of arabinose and galactose, both of which reduce Fehling's solution.

This combination of cytohydrolytic enzymes from the mycelium of *Trametes subrosea* is the explanation of the brown rot resulting from its wood-destroying activity in the peach and prune wood. Doubtless, also, the presence of emulsin makes available more food which exists in the wood in the form of glucosides. This ability of this fungus to make available as food these substances in rapidly grown orchard trees may be a partial explanation for the very rapid and devastating advancement of such forest parasites on these trees. For, it is a recognized fact that such forest-tree wound parasites as those represented by *Trametes subrosea* attack orchard trees more energetically than they seem to attack forest trees. Whether carbohydrates are more plentiful or more easily made available in the rapidly grown wood of orchard trees is not known.

DAMAGE TO ORCHARD TREES

The damage done by this heart rot is not easily estimated, because of the inability to estimate the cost of bring trees into bearing and the cost of replacing trees made decrepit through the influence of heart rot produced by *Trametes subrosea*. Other difficulties arising in such estimates of damage are the many different phases of the damage. In the first place the wood decay is not limited to the heart wood. Especially is this true of prune trees. After a tree has been affected for a number of years, the decayed cylinder of wood increases in diameter until the amount of sapwood is relatively small. In such cases it is not uncommon to find (especially if one side of a tree has been affected by winter injury) long sunken areas where the decayed wood extends to the bark. These may extend well up into smaller branches. This encroachment upon the sapwood materially arrests the desired increment of bearing wood on the branches affected, or on all of the branches above such a sunken area on the trunk.

Wood decay produced by *Tramates subrosea* has a marked influence on the neighboring sapwood. In the healthy sapwood of both peach and prune, wood tyloses are found scatteringly in the larger vessels of the spring wood only, none having been found in the smaller vessels of the summer growth. Where only 3 to 6 annual rings of sapwood separate the decayed cylinder within from the bark, tyloses were extremely numerous in the larger vessels of the spring wood in rings 2 or more years old. In fact, in prune wood especially, vessels of spring wood have been found nearly filled with tyloses, and tyloses were also numerous in the vessels of the summer wood. Where several annual rings separate the decayed heart and the last year's wood growth, the toxic influence of the decayed wood on the production of tyloses in vessels 2 or 3 years old is nearly as great as where

the fungus is nearer. Stevens (*3*) has described a similar condition in Catalpa sapwood influenced by the decay produced by *Polystictus versicolor* in closely neighboring tissue.

Such disturbances surely influence the normal physiology of a tree, bringing about a weakened framework, increasing the mortality of crop-producing wood, and causing increased susceptibility to winter injury. There are also indications that the lack of water conduction in wood having such an abundance of tyloses has a direct contributary bearing on the physiological disturbances in prune trees known as leaf roll, June drop of fruit, and early yellowing and dropping of leaves.

An orchard survey was made to ascertain just how much damage is done by the wood decay caused by *Trametes subrosea*. Out of 1,261 prune trees in orchards having average horticultural care, 1,212 showed heart rot. Of these, 885 were affected by *T. subrosea*. That is, 73 per cent of the heart rot found in the orchards is caused by *T. subrosea*. Of the 885 trees affected with this disease, 619 (or about 70 per cent) had pruning cuts 2 inches or more in diameter. These pruning cuts had not been treated with an antiseptic wound dressing.

In Douglas County, Oreg., 100 trees were examined in each of two neighboring orchards of Italian prunes. One orchard showed extremely good care, all pruning cuts of about 1.5 inches in diameter having been treated with some type of asphaltum wound dressing. The other orchard showed lack of care in the protection of wounds. In the orchard showing good care, 91 per cent of the trees showed no evidence of wood decay, although 82 per cent of them had large pruning cuts. On the other hand, of the 100 trees in the orchard receiving meager care only 7 per cent were sound and all had large pruning cuts. Of the 93 trees showing wood decay, 78, or 83.9 per cent, had the brown-pocket rot due to *Trametes subrosea*, while all of the 9 trees showing decayed wood in the other orchard were affected by this organism.

In some localities within this active range of the fungus, vigorously growing older orchards do not seem to be affected by the heart rot. On river bottom land, such as that in the Days Creek Valley, Douglas County, Oreg., and in Clarke County, Wash., prune trees grow to large size. The lack of heart rot in many orchards of some of these communities seems to be related to the system of pruning adopted by the grower. In brief, heart rots are less prevalent in orchards where the system of pruning used does away with all pruning on the main leader branches and trunk, particularly where trees are so trained in early years that the removal of large branches is unnecessary later.

PREVENTIVE MEASURES

To prevent the wood decay of stone-fruit trees caused by *Trametes subrosea*, the measures usually suggested for prevention of infection by wood-destroying fungi are recommended, i. e., pruning properly so that the wound will heal in the shortest possible time, and treatment with a wound dressing. The writer has had success with a Bordeaux paint made up by mixing linseed oil into a Bordeaux dust until a thick, smooth paint is formed. Preferably, however, it is better to educate growers to a pruning system whereby large

pruning cuts which need treatment are unnecessary or reduced to a minimum.

SUMMARY

Trametes subrosea Weir causes a brown-pocket heart rot of peach and prune trees in orchard districts from central California to British Columbia. The brown rot has been demonstrated to have been produced by the cytohydrolytic enzymes ligninase, cellulase, and hemicellulase. Emulsin is also present in the mycelium of *T. subrosea*, making available the products from glucoside digestion in the wood.

The economic losses due to this decay in orchard trees are large but difficult to estimate. Also, the damage to the diseased tree is not limited to the heartwood, since the disease encroaches upon the sapwood, cutting down its volume and actually destroying the water conducting power of the remaining sapwood by the stimulation of the growth of tyloses in the xylem vessels, upsetting the physiological balance in the tree, and giving rise to "drouth effects" in the affected portions of the tops.

A survey in prune orchards showed 73 per cent of the heart rot to be produced by *Trametes subrosea*, and demonstrated that the presence of this heart rot is related to large, unprotected pruning cuts.

Preventative measures are (1) proper pruning to facilitate healing, (2) treating the wounds with an antiseptic dressing, and preferably (3) the use of a system of pruning such that large pruning cuts are unnecessary or are reduced to a minimum.

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THE WATER-SOLUBLE CONTENT OF CALCIUM AND PHOSPHORUS IN CABBAGE [1]

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INTRODUCTION

In the preservation of plant materials by natural fermentation processes, such as the making of silage and sauerkraut, the quantity of nutrients expressed from the plant tissue must to a large extent determine the rate at which the fermentation proceeds. It is not merely sugar but also soluble nitrogen and salts that are involved in the development of the acid-producing bacteria. Of the mineral elements in the expressed juice, it is probable that calcium and phosphorus are among the most important.

For this reason it is of interest to know what percentage of these mineral elements is soluble in water. The present study is concerned with the soluble calcium and phosphorus of cabbage, the ratio which each bears to the total quantity of the particular element, and the variation of each with the type of soil and the maturity of the plant. Incidentally, the data for 1925 will be compared with those reported in a previous paper (*8*) [2] showing the variations in the total calcium and phosphorus of 18 samples of cabbage grown during the season of 1924 in various parts of the United States.

EXPERIMENTAL PROCEDURE

Most of the cabbage was grown on two types of soil; an upland sandy loam which had been cropped for many years and an alkaline marsh soil which had been drained and kept under cultivation for about 10 years.

The cabbage was grown for kraut-making purposes, and from 50 to 60 heads were taken at each time of harvesting. The heads were stripped of the outer green leaves and cut into long shreds in a factory kraut cutter. From 100 to 125 pounds of shredded cabbage were obtained each time in this way. After mixing the whole quantity thoroughly to obtain uniformity, a sample of 400-gm. weight was moistened with ether, ground in a large mortar with sharp sand, and repeatedly extracted with fresh portions of water. The cloudy extract was filtered through paper pulp on a Büchner funnel and came through perfectly clear. After washing with several portions of water, the filtrate and washings were made up to 2,000 c. c. The above procedure is essentially that described by Tottingham, Schulz, and Lepkovsky (*9*) for extracting the nitrogenous constituents from plant cells. Three hundred c. c. aliquots were taken for calcium determinations and 100 c. c. for phosphorus.

Other portions of the cabbage were dried for the determination of moisture, calcium, and phosphorus. Moisture was ascertained by

[1] Received for publication May 1, 1926; issued October, 1926. Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 699.

drying at 100° C. Both total and soluble calcium were determined by the McCrudden (*3*, *4*) method as used when iron is contained in the sample. The two forms of phosphorus were obtained by the volumetric method of the Association of Official Agricultural Chemists (*2*, *p. 3*).

In Table 1 are given the chief facts regarding the variety, date of harvest, moisture and nitrogen content of the cabbage, and the type of soil on which the cabbage was grown. The samples are arranged so as to bring those from the same plot of ground together in one group, and the samples within the group are listed according to the date of harvest. The first sample was taken on September 16 when the cabbage was very green and not fully grown. Owing to unexpected freezing weather, the samples taken on November 11 were frozen solid and had been in this condition for four to five days before they were brought in from the field.

As was noted in a previous paper (*6*), the nitrogen content increased with maturity. This is particularly marked in the Miami silt-loam samples.

TABLE 1.—*Description of cabbage samples*

Sample No.	Variety of cabbage	Type and location of soil plot	Date of harvest	Moisture	Nitrogen
				Per cent	*Per cent*
349	All Season	Alkaline marsh, Madison, Wis.	Sept. 29, 1925	93.8	3.87
352	do	do	Oct. 23, 1925	93.6	3.33
355	All Season (frozen)	do	Nov. 11, 1925	93.9	3.86
350	All Season	Miami silt loam, Madison, Wis.	Oct. 6, 1925	93.1	3.09
354	do	do	Oct. 28, 1925	92.9	3.41
356	All Season (frozen)	do	Nov. 11, 1925	93.8	3.94
347	Glory	Clyde silt loam, Racine, Wis.	Sept. 16, 1925	93.2	1.92
348	All Head	do	do	93.3	2.00
351	All Season	do	Oct. 15, 1925	93.2	2.95
353 [a]	do	do	Oct. 28, 1925	92.9	2.95
357	do	Franksville, Wis.	Nov. 5, 1925	93.5	3.16
358	All Season (frozen)	do	do	93.8	3.38
Average				93.4	3.16

[a] Same lot of cabbage as No. 351, stored 13 days.

VARIATIONS IN TOTAL AND WATER-SOLUBLE CALCIUM

In Table 2 are given the date for the calcium content of the samples. The percentage of calcium in the fresh vegetable ranged from 0.038 to 0.053 with an average of 0.046. In the paper previously reported one sample ran as low as 0.029, but the other 17 samples fell within the limits of 0.038 and 0.056 per cent and averaged 0.043 per cent. The results for the two years 1924 and 1925 are unexpectedly close to one another, especially when it is considered that the samples for the two years were grown on different types of soil and in different parts of the country. The marsh samples contained approximately one-third more calcium than the upland samples. This excess is probably related to the higher calcium content in the soil. Both of these sets of samples showed a drop in the calcium content of the second cutting as compared to the first; while the third cutting in both cases not only regained this difference, but slightly exceeded the first cutting in percentage of calcium. The Racine samples also showed a tendency toward higher calcium in the late samples than in the early ones. Less importance can be attached to these figures, however, since all the samples were not of the same variety of cabbage.

TABLE 2.—*Total and water-soluble calcium of cabbage*

Sample No.	Calcium in dry matter		Percentage of total calcium extracted by water	Calcium in fresh vegetable	Sample No.	Calcium in dry matter		Percentage of total calcium extracted by water	Calcium in fresh vegetable
	Total	Soluble				Total	Soluble		
	Per cent	*Per cent*		*Per cent*		*Per cent*	*Per cent*		*Per cent*
349	0.81	0.49	60	0.050	348	0.57	0.37	65	0.038
352	.80	.46	57	.051	351	.63	.41	65	.043
355	.86	.36	42	.053	353	.65	.32	50	.046
350	.65	.44	67	.042	357	.70	.44	63	.046
354	.60	.35	58	.043	358	.73	.45	61	.045
356	.75	.46	61	.047					
347	.66	.45	68	.045	Average	.70	.42	60	.046

As the cabbage matures the percentage of water-soluble calcium decreases. All the samples show a change from soluble to insoluble forms of calcium as the season advances, but the marsh samples manifest this change to the most marked degree. This cabbage contained 60 per cent of the total calcium in a water-soluble form on September 29 and only 42 per cent on November 11. The effect of storage on water-soluble calcium is shown by samples 351 and 353. Storage for 13 days reduced the water-soluble calcium from 65 to 50 per cent of the total.

VARIATIONS IN THE TOTAL AND WATER-SOLUBLE PHOSPHORUS OF CABBAGE

In Table 3 are given the data for the phosphorus content of the samples. The maximum percentage in any sample of fresh vegetable is 0.036, the minimum is 0.023 and the average is 0.028. The variations in the 1924 samples were somewhat greater, extending from 0.017 to 0.037 per cent. This is not surprising since the 1924 experiment included a larger number of samples from more widely separated sources. The average for the earlier year, 0.024 is but slightly less than that for 1925. There appears to be a slight increase in total phosphorus as the crop matures, but the increase is too slight to warrant any definite conclusion.

TABLE 3.—*Total and water-soluble phosphorus of cabbage*

Sample No.	Phosphorus on dry basis		Percentage of total phosphorus extracted by water	Phosphorus in fresh vegetable	Sample No.	Phosphorus on dry basis		Percentage of total phosphorus extracted by water	Phosphorus in fresh vegetable
	Total	Soluble				Total	Soluble		
	Per cent	*Per cent*		*Per cent*		*Per cent*	*Per cent*		*Per cent*
349	0.40	0.26	65	0.025	351	0.41	0.30	73	0.028
352	.38	.24	63	.024	353	.40	.20	50	.028
355	.44	.18	41	.027	357	.42	.27	64	.027
350	.43	.34	79	.030	358	.37	.28	76	.023
354	.50	.30	60	.036					
356	.53	.21	39	.033	Average	.43	.26	61	.028

As in the case of calcium, the soluble phosphorus decreases as the cabbage matures. In the Madison sets of samples the soluble phosphorus decreased from one-third to one-half of what it was when the earliest sample was taken. As shown by a comparison of samples 351 and 353, storage reduced the solubility of the phosphorus even more than it did that of calcium. Additional data are needed, however, to determine whether this is the general effect of storage.

The figures for soluble calcium and phosphorus are of the same order of magnitude as those which have been reported for plant materials by other investigators.

Ames and Boltz (*1*) found that 75 per cent of the phosphorus and 40 per cent of the calcium in alfalfa could be extracted by water. In a later paper Osborne, Wakeman, and Leavenworth (*5*) reported that 69.3 per cent of the inorganic matter of alfalfa was soluble in water, and that 49.2 per cent could be extracted by grinding and pressing the green plants. Of the latter 16.7 per cent was calcium and 5.5 per cent phosphorus.

In a study of the loss of mineral elements by various methods of cooking, Peterson and Hoppert (*7*) found that from summer cabbage 72 per cent of the calcium and 60 per cent of the phosphorus was extracted by boiling water.

In Table 4 are summarized the data for the two years 1924 and 1925. Although samples from the same year may vary from 50 to 100 per cent, the averages for the two years are very near together. In all but one case the averages for the two years differ by less than 10 per cent. The moisture figures for the two years are almost identical.

TABLE 4.—*Two-year comparison of the average composition of cabbage, based upon 18 samples for 1924 and 12 for 1925*

Constituent and range	1924	1925	Constituent and range	1924	1925
Calcium:	*Per ct.*	*Per ct.*	Total nitrogen:	*Per ct.*	*Per ct.*
Maximum	0.056	0.053	Maximum	0.24	0.24
Minimum	.029	.038	Minimum	.15	.13
Average	.043	.046	Average	.19	.21
Phosphorus:			Moisture:		
Maximum	.037	.036	Maximum	93.9	93.9
Minimum	.017	.023	Minimum	91.0	92.9
Average	.024	.028	Average	92.6	93.4

SUMMARY

The percentage of calcium for 12 samples of cabbage ranged from 0.038 to 0.053 with an average of 0.046. On an average, 60 per cent of this calcium was soluble in water. The percentage of water-soluble calcium was highest in immature cabbage and decreased as the plant grew older.

The figures for phosphorus in these same samples varied from 0.023 to 0.036 per cent and averaged 0.028 per cent. The percentage of phosphorus soluble in water, 61, was almost exactly the same as that for water-soluble calcium. The solubility of the phosphorus decreased as the season advanced.

The average figures for these two elements for 1925 differ by less than 10 per cent from those obtained in 1924 from 18 samples of cabbage grown in some of the important cabbage-producing regions of the United States.

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